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Histopathology of the adrenal cortex and adenohypophysis in cattle with mucosal disease

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HISTOPATHOLOGY OF THE ADRENAL CORTEX
AND ADENOHYPOPHYSIS IN CATTLE WITH MUCOSAL DISEASE

by

Charles Elmer Whiteman

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Veterinary Pathology

Approved:

Signature was redacted for privacy.

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Ames, Iowa

1960

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INTRODUCTION*

In 1953 Ramsey and Chivers reported a disease syndrome in Iowa cattle to which they tentatively gave the name mucosal disease**. This descriptive name indicated that lesions involving the mucosa of the digestive tract were prominent upon post-mortem examination of affected animals.

Reports of similar disease syndromes were previously recorded and additional reports appeared subsequently in the literature. Many of the reports probably described the same, or closely related, disease syndromes with some variation of symptoms and lesions. This group of diseases is often referred to as "the mucosal disease complex," or, more loosely, as "mucosal disease." Common names have been applied to certain members of this complex. An arbitrary listing of some of the syndromes that could be included in the mucosal disease complex follows:

X-disease of cattle-Saskatchewan. (Childs, 1946)

Virus diarrhea-New York. (Olafson et al., 1946)

Mucosal disease-Iowa. (Ramsey and Chivers, 1953)

Virus diarrhea-Indiana. (Pritchard et al., 1956)

Mycotic stomatitis. (Hutyra et al., 1946)

"Muzzle disease." (Hollister et al., 1956)

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**In this thesis the term mucosal disease refers specifically to the disease described by Ramsey and Chivers.

An infectious ulcerative stomatitis of cattle. (Pritchard et al., 1958)

Certain enzootic diseases of cattle also present lesions of the digestive tract that resemble those seen in the mucosal diseases. Prominent among these diseases are hyperkeratosis, malignant head catarrh, and coccidiosis.

The costly foreign disease, rinderpest, has a very striking similarity of lesions when compared with some of the members of the mucosal disease complex. This similarity could result in rinderpest being introduced and widely disseminated in the United States before being identified. To prevent this possibility it is desirable that the mucosal disease complex be investigated and the various entities characterized so that a prompt reliable diagnosis can be made.

Complete descriptions of the lesions of the various mucosal diseases have not been completed. However, considerable work has been done on some entities. Ramsey (1956), working with the Iowa type of mucosal disease, described many of its gross and microscopic lesions. His work, although extensive, did not include a detailed study of the adrenal cortex or adenohypophysis.

Before considering the adrenal cortex and adenohypophysis in cattle with mucosal disease one should note the voluminous literature on the marked response of these glands to stress in laboratory animals. Responses have been noted following such stress factors as forced exercise, exposure to temperature extremes, burns, exposure to natural or synthetic estrogens, inanition, anoxia, toxic substances, high protein

diets, and the course of infections. The most frequently reported response has been adrenal cortical hypertrophy. It appears that pituitary studies in stress response have been made less frequently.

Insofar as the author is aware no comprehensive study has been made of the adrenals and adenohypophyses of cattle subjected to a variety of non-specific stress producing factors or agents. It seems reasonable to hypothesize that histopathological changes may occur in the adrenals and pituitary glands of cattle due to the stress of the natural course of mucosal disease, or as a result of the direct action of an unknown etiological agent or agents. It is the purpose of this research problem to determine whether histopathological changes are present in the adrenal cortices and adenohypophyses of cattle with mucosal disease and to describe such changes as may be observed.

REVIEW OF LITERATURE

The Adenohypophysis and Adrenal Cortex
in the Mucosal Disease Complex

The adenohypophysis appears not to have been investigated in any of the syndromes of the mucosal disease complex.

Rooney (1957) reported on a bovine mucosal-type disease. Of the literature reviewed for the present study, Rooney's report contained the only recorded observation on adrenal alterations in a disease possibly related to Iowa mucosal disease. An evaluation of lipid accumulations in the adrenals of cattle studied by Rooney showed all possible variations from no demonstrable vacuoles to marked widespread vacuolation. Many of the clinical features and other post-mortem lesions of the mucosal-type disease described by Rooney differed markedly from those seen in Iowa mucosal disease.

The Adenohypophysis and Adrenal Cortex
in Disease and Stress Conditions in Laboratory Animals

Regardless of the paucity of material on adenohypophyseal and adrenal changes in the mucosal disease complex, there is a large body of literature on changes in the adenohypophyses and adrenals of laboratory animals under disease and stress conditions. To properly evaluate the lesions of the pituitary-adrenal axis it must be understood that the physiology of the adrenal cortex is intimately concerned with that of the adenohypophysis. Alterations in either the adenohypophysis or the adrenal cortex may result in a compensatory change in the other structure.

The most frequently reported adrenal response to disease or stress was adrenal cortical hypertrophy. Tepperman, Engel and Long (1943a) reviewed adrenal cortical hypertrophy and Soffer (1946) discussed it in his book on diseases of the adrenals. Tepperman et al. (1943a) stated that hypertrophy of the adrenal glands had been described in a bewildering variety of circumstances. Some of the factors that they reported as leading to adrenal cortical hypertrophy included forced exercise, temperature extremes, burns, natural and synthetic estrogens, inanition, anoxia, toxic substances, high protein diets, and infections. Many of the factors that produced adrenal cortical hypertrophy in laboratory animals were characterized by increased protein catabolism. Some of the factors were of special interest because of the possible bearing on adrenal alterations present in Iowa mucosal disease.

Estrogens

Bourne and Zuckerman (1940) noted that the adrenal glands of rats fluctuated in size in a period corresponding to the oestrous cycle. Threshold doses of oestrone in ovariectomized rats led to a marked increase in the size of the adrenal cortex, whereas large doses had a lesser stimulating effect. Evidence indicated that the influence of oestrogenic stimulation on the adrenals was mediated through the anterior pituitary. This opinion was reached also by Fry, Miller and Long (1942). Ingle (1941) observed a marked hypertrophy of the adrenal cortices in rats treated with stilbestrol and other estrogens. James and Nelson (1942), in studying the influence of diethyl-stilbestrol on

carbohydrate metabolism, observed that the adrenals and pituitaries of rats were increased in size. The increase was greater in females than in males.

Inanition

Jackson (1915) and Selye (1936) made early observations on the increased weights of rat adrenals as the result of underfeeding and starvation. Similar observations were made later by Sarason (1943) and by Mulinos, Pomerantz and Lojkin (1942). D'Angelo, Gordon and Charipper (1948) stated that the enlargement of the adrenals in guinea pigs was a characteristic response to deprivation of food. In those guinea pigs adrenal hypertrophy involved the zona fasciculata and zona reticularis, whereas the zona glomerulosa atrophied. D'Angelo, Gordon and Charipper (1948) also noted a moderate loss of pituitary weight in starving guinea pigs.

Anoxia

Hypertrophy of the adrenals in animals exposed to low air pressure was reported by Armstrong and Heim (1938) and by Langley and Clark (1942). The physiological explanation of adrenal adjustment to anoxia received the attention of Evans (1934, 1936). Adaptation to anoxia apparently demands, as one factor, an increase in protein catabolism. Protein catabolism is related to certain adrenocortical hormones. Adrenal activation necessary for physiological adjustment to anoxia may be associated in some cases with adrenal cortical hypertrophy.

Toxic substances

Adrenal hypertrophy was described following the administration of a wide variety of unrelated toxic substances, and numerous references were given by Tepperman, Engel and Long (1943a). Lewis and Page (1948), working with botulinus and diphtheria toxins, observed that the minimum lethal dose of these toxins for rats was much greater in normal rats than in adrenalectomized rats. The great variety of toxic substances, or toxins, that produce an increase in adrenal size suggests that many of those substances produce their effects through a common mechanism. It appears likely that the list of toxic substances that might produce adrenal cortical hypertrophy could be extended through further research on a plethora of chemicals now used in agriculture as insecticides, fertilizers, and weed or brush killers.

High protein diets

Tepperman, Engel and Long (1943b) studied the effect of high protein diet on size and activity of the adrenal cortex in the albino rat. On the basis of the experimental data on animals receiving high protein diets, and on the basis of a survey of the literature, it was suggested that some event or series of events, occurring during the catabolism of protein, may serve as a stimulus for the hypertrophy of the adrenal cortex. More recently, Engel (1951) suggested that in the presence of stress there is apparently an intense need for protein catabolism. Adrenal cortical hypertrophy may be a part of the body response necessary to provide additional hormone for protein catabolism, but this point is

unclear.

Infections

Anderson (1935), in the course of other work, observed adrenal hypertrophy in rats from accidental infections of the lungs and middle ears. Olitzki, Avinery and Koch (1942) observed adrenal cortical enlargement and hemorrhage in guinea pigs in response to the injection of numerous killed bacterial vaccines. Deanesly (1931) described adrenal hypertrophy following either natural infection or inoculation of killed bacteria into mice. Clinical and experimental data have revealed that the adrenals were frequently enlarged in animals with infections. This lack of specificity of adrenal cortical hypertrophy suggests that it is not infection itself that produces adrenal hypertrophy.

The General Adaptation Syndrome

Selye (1946) published a detailed account of the general adaptation syndrome. Other useful publications (Selye, 1944, 1952) were published also. Within the framework of the general adaptation syndrome concept the previously discussed causes of adrenal cortical hypertrophy, and other causes, were postulated as nonspecific stimuli or "stressors." It was proposed that these stimuli regularly stimulated certain adaptive responses within the living laboratory animals to which they were applied. The sum of the nonspecific adaptive responses was referred to as the general adaptation syndrome. Pituitary functions, especially the production of adrenocorticotrophic hormone, were related to adrenal

hypertrophy in the general adaptation syndrome.

The Adenohypophysis and Adrenal Cortex
in Diseases of Cattle and Sheep

So-called metabolic diseases of cattle and sheep

Ketosis and parturient paresis in dairy cows

Ketosis and parturi-

ent paresis of cows have been referred to as "metabolic diseases," as has Domsiekte, pregnancy disease, of sheep. The clinical picture in ketosis or in parturient paresis bears little similarity with the clinical picture of mucosal disease. Nevertheless, some interesting histopathological comparisons can be made between these two conditions and mucosal disease.

Shaw and various co-workers (1948, 1949, 1950, 1951) investigated both spontaneous ketosis and fasting ketosis in cows. In toto, these workers reported a number of histopathological alterations that occurred in the adrenal and in the adenohypophysis. The alterations in the adrenal included enlargement of the gland, the presence of 'degenerative areas of the adrenal cortex' (most marked in the zona glomerulosa), a high total fat content, and marked fatty infiltration.

Shaw, Hatziolos and Leffel (1950) listed lesions noted in the pituitary-adrenal system and referred to 'regressive changes of the anterior lobe of the pituitary gland.' In the same year Hatziolos and Shaw (1950) also reported atrophy and fatty degeneration of the anterior lobe of the pituitary. Furthermore, they made a tentative proposal that ketosis in cows is basically a disease of the so-called adaptation

syndrome brought on by various stresses during a period when the organism was overworked.

Bell and Weber (1959) made a comparative study of lipid accumulations in the adrenal glands of certain classes of dairy heifers and cows. The study was undertaken to test the hypothesis that the adrenal glands of lactating and pregnant cows undergo definite cytological and morphological changes similar to those seen in the glands of ketotic cows. Bell found that although the lipid content of the glands from the pregnant lactating cows appeared to be much less than that seen in cows with ketosis and other parturient disease, the pattern of distribution of the lipid material resembled that seen in cows with ketosis and other postparturient conditions.

Garm (1950) studied the endocrine glands from eleven cases of parturient paresis and two cases of parturient eclampsia in cows. The adrenals were remarkably large and the cortices were hypertrophied and had a yellow color. Staining with Sudan IV and Sudan black showed the epithelial cells in all cortical zones to be filled with lipoid substances. The lipoids all reacted negatively to the Schultz cholesterol test.

In the pituitaries of the same animals Garm (1950) also noted abundant colloid substance in the pars anterior and in the residual lumen. The pars posterior showed a remarkable invasion of basophils. Differential cell counts of the various cell types were not made, but it appeared that there was an increased number of basophils and chromophobes.

Blood changes observed by Garm (1950) included eosinopenia, lymphopenia, and neutrophilia. He concluded that the changes in the blood,

adrenals, and hypophysis indicated an increased pituitary-adrenal cortical activity. Garm suggested that the syndromes of parturient paresis and parturient eclampsia in cows should be included in the group of diseases to which Selye has applied the term "diseases of adaptation."

Holcombe (1953) studied the excretion of reducing corticoids and neutral steroids in the urine of cows with parturient paresis and eclampsia. He interpreted certain variations in the urinary content of those substances as a symptom of extreme adrenal cortical exhaustion.

Domsiekte in sheep In the course of a study on Domsiekte, pregnancy disease of sheep, Groenewald, Graf, Bekker, Malan and Clark (1941) made some interesting observations on the adrenals of sheep having this disease. Upon microscopic examination of lipid-stained adrenal sections from pregnant normal sheep, a fine sprinkling of evenly sized small red droplets was observed. It was noticeable that the zona glomerulosa contained very little fat, if any. In pregnant Domsiekte-affected adrenals large irregularly sized fat droplets were encountered in the majority of the cells, and the zona glomerulosa was more affected than the rest of the cortex. The adrenal cortices of pregnant sheep were observed to be far more susceptible to lipid accumulation than the adrenal cortices of non-pregnant ewes. The tendency of the adrenal cortices of pregnant sheep to accumulate lipids may be involved in the pathogenesis of Domsiekte.

Nymphomania in cows

In the course of extensive work with bovine nymphomania Garm (1949) studied the pituitaries and adrenals of nymphomaniac cows and compared them with similar glands from normal cows. It appeared to Garm that the mean of the sum of acidophils and the mean of the total percentual number of basophils and chromophobes was nearly the same in both groups of cows.

Garm (1949) theorized that in the pathogenesis of nymphomania the primary dysfunction of the anterior pituitary was in the increased production of follicle stimulating hormone and corticotrophic hormone by the basophilic cells. The increased production of these hormones led to the development of Graafian follicles in the ovaries and hypertrophy of the adrenal cortex. Ovulation did not occur owing to failure of the basophils to produce a sufficient amount of luteal hormone. Estrogens from the persisting Graafian follicles stimulated the pituitary to an increased production of corticotrophic hormone and at the same time directly stimulated further adrenal cortical hypertrophy. Adrenal cortical hypertrophy led to increased production of the salt retaining adrenal hormones that were influential in preventing follicular atresia.

In all types of nymphomania there was a statistically highly significant increase in the absolute and relative weight and volume of the hypophysis, and in the width of the adrenal cortex. In the adrenal cortex the zona glomerulosa appeared to be wider than in normal cows. Garm was unable to draw definite conclusions concerning the amount and distribution of adrenal lipids.

Malignant head catarrh

Both Stenius (1952) and Plowright (1953) noted alterations in the adrenals of cattle with malignant head catarrh. Stenius (1952) noted a diffuse fatty infiltration of parenchymal cells in the zona glomerulosa and fasciculi corticales. Plowright (1953), in discussing the pathology of infectious bovine malignant catarrh in cattle and rabbits, referred to lesions in the parenchymatous organs, including the adrenals. He referred to those lesions as interstitial lymphoid-macrophage accumulations. In cattle the lymphoid-macrophage accumulations were capsular, cortical (especially in the zona glomerulosa) and medullary. Widespread degeneration of the secreting cells was present, often with small areas of diffuse necrosis. Cellular degeneration was accompanied by the appearance of large free macrophages.

The experimental production of adrenal lesions in calves

Weber, Pritchard, and Sellers (1956), working with calves, produced adrenal cortical alterations by the injections of various concentrations of sodium and potassium salts. Similar alterations were produced in calves drenched with sodium acetate while water was simultaneously restricted. Oil red O-stainable lipids and cholesterol were markedly increased in quantity in the zona glomerulosa. The thickness of the zona glomerulosa and cell size within the zona glomerulosa remained unchanged by this treatment.

Weber, Bell, and Sellers (1958) produced lesions in the zona fasciculata and zona reticularis of calves by the administration of DDD,

a chlorinated hydrocarbon. A prominent feature of the lesions was the accumulation of coarse lipid droplets in the cells of the zona fasciculata and zona reticularis. Continued administration of DDD over specified periods of time also produced a pattern of cholesterol accumulation that followed the pattern of lipid appearance.

Bovine adenohipophyseal functional cytology and alterations that occur with stress

Jubb and McEntee (1955a) reviewed the literature on adenohipophyseal functional cytology in cattle. Jubb and McEntee (1955b) also published work on the architecture and cytology of the bovine pituitary with special reference to basophil cell function. In the latter publication Jubb and McEntee divided the basophils, cells containing granules stainable by the periodic acid-Schiff method, into beta and delta types. They reported that beta cells were related to thyrotrophic hormone production, and delta cells were related to gonadotrophic activity, especially luteinizing hormone release. The total percentage of basophils in the castrated animal was approximately five percent less than in bulls. This difference was attributed to the greater percentage of delta-type basophils in bulls. In the castrated bovine and freemartin the delta cells were smaller in number and the content of the periodic acid-Schiff positive granules was markedly diminished.

In their work on the architecture and cytology of the bovine pituitary Jubb and McEntee (1955b) also noted a degranulating response of the acidophils to prolonged nonspecific stress. The degranulation response gave no evidence of specificity and the authors remarked on the uniformi-

ty of the acidophilic response to an appropriate stimulus. A degree of degranulation of acidophils was noted as a primary or secondary response to such factors as thyroidectomy, ovulation, the presence of multiple metastatic abscesses, and poisoning with highly chlorinated naphthalenes. Hypergranulation of acidophils was observed in four genetic dwarf Herefords, in cows with cystic ovaries, and in three cows given parenteral estrogens.

The Origin of Adrenocorticotrophic Hormone (ACTH)

Although adrenocorticotrophic hormone is primarily concerned with adrenocortical hypertrophy, there appears to be no agreement as to the precise cellular origin of adrenocorticotrophic hormone (ACTH) from the parenchymatous adenohypophyseal cells. D'Angelo, Gordon and Charipper (1948) studied the effect of inanition on the anterior pituitary-adrenocortical interrelationship in the guinea pig. They observed in starving guinea pigs that acidophils lost their granulation, decreased in size, and became less numerous, whereas basophils apparently increased in number and were especially well developed. They interpreted adrenal cortical hypertrophy as being due to adrenotropin secretion from the basophils of the anterior pituitary.

Marshall (1951) adapted the fluorescent antibody technique to the localization of protein antigens in cells and tissues. The method was applied specifically to the localization of adrenocorticotrophic hormone (ACTH) in the pituitary gland. A fluorescent antibody solution, prepared from an adrenalectomized rabbit that was immunized against porcine ACTH,

stained selectively the cytoplasm of basophilic cells of the porcine pituitary. This could be interpreted to indicate that ACTH originated from basophils in swine.

Herlant (1952, 1953a, 1953b) showed that acidophilic granules separated from the pituitary cells of sheep by centrifugation contained ACTH activity for rats. Barnett, Ladman, McAllaster and Siperstein (1956) extracted glycoprotein hormone from the pituitary glands of rats so that the cytoplasm of basophils was lacking in stainable material, whereas the cytoplasm of the acidophils largely remained intact. Bioassay of similarly treated pituitaries (Barnett, Siperstein and Josimovich, 1956) revealed the presence of ACTH. According to histochemical staining results, the ACTH could not have been in the basophils. This work suggested that ACTH was produced in the acidophils.

Ladman and Barnett (1956) studied the alterations in chemically demonstrable protein bound sulfhydryl (SH) and disulfide (S-S) groups in the cells of the anterior pituitaries of rats that had been subjected to various experimental endocrine conditions. The alterations in the staining of the acidophils following acute or chronic stress, or adrenalectomy, indicated to them that the production of ACTH was connected with the acidophils. These workers hypothesized that ACTH is associated with a carrier protein that is high in S-S groups.

MATERIALS AND METHODS

The adrenals from 42 cases of mucosal disease were available for this study. The pituitaries of 25 of those animals were also available. The adrenals and/or pituitaries of 18 comparable, apparently-normal cattle* were secured at the Iowa State University meats laboratory and served as controls. Of the mucosal disease tissues used for this study, a small number was secured before the research problem was selected. They had been fixed in 10 percent formalin. Additional tissues were added as cases came into the Iowa State University veterinary clinic for treatment or diagnosis.

Adrenals were collected from 23 mucosal disease affected cattle that were sacrificed. Twenty of those cattle progressed to a moribund condition, euthanasia was performed using 110 volts of electricity, and tissues were collected immediately during the routine necropsy. The other three cattle received euthanasia before reaching extremis so as to study the early stages of mucosal disease. Nineteen of the 42 pairs of adrenals came from animals that died during the natural course of mucosal disease. Tissues were collected from those animals within one to three hours after death. Adrenals and pituitaries collected within three hours of death were free of identifiable post-mortem change.

The adrenals were removed and the attached body fat was cut away carefully with a sharp knife and scissors. The glands were then weighed

*The term normal cattle will be used in the text hereafter and will mean that the animals were free of symptoms of disease.

(Tables 1 and 2) and sectioned transversely to their long axis. The left adrenal was used as a source of tissue for study because its structure permitted the taking of sections from the narrowed neck-like area near the middle of the gland. This procedure resulted in comparable sections from various animals.

Adrenal tissues were fixed at room temperature in buffered formalin (Armed Forces Institute of Pathology, 1957), calcium-cadmium-formol (Baker, 1944), and mercury-formol-saline (Dawson and Friedgood, 1938). After 24 hours any necessary additional trimming was done and the tissues were placed again in freshly prepared fixative. Tissues fixed in calcium-cadmium-formol were then stored in the fresh fixative at refrigerator temperature until a sufficient number accumulated to warrant doing frozen section preparations. This period of storage varied from seven to 150 days. Tissues that had been fixed in buffered formalin were allowed to remain in that fixative for a minimum of three days, and thereafter were stored in 70 percent alcohol. The tissues fixed in mercury-formol-saline were fixed for three days, washed overnight in running tap water, and stored in 70 percent alcohol.

Tissues stored in 70 percent alcohol were dehydrated consecutively through 70 percent, 95 percent, and absolute ethyl alcohol. After decanting the absolute alcohol the tissues were immersed in chloroform for 12 hours to remove all traces of alcohol and to clear the tissues. Infiltration with paraffin was accomplished by placing the tissues first in closed dishes of chloroform-paraffin (1:3) for two hours, and secondly in pure paraffin for two to four hours, as needed for proper infiltration.

Table 1. Adrenal and pituitary weights of 18 Iowa mucosal disease affected cattle^a

Animal number	Age ^b (months)	Sex ^c	Breed ^d	Body weight ^b (kg)	Adrenal weight ^e (gms)	Pituitary weight (gms)
22	5	S	H	168	12.200	.875
24	7	S	H	193	17.500	1.000
121	8	F	SH	261	17.710	1.090
131	8	F	A	182	18.750	1.130
35	8	F	Gue	182	19.800	1.200
69	9	S	H	272	16.600	1.100
130	10	F	H	182	20.260	1.200
27	10	F	Hol	273	31.300	1.000
118	11	S	H	261	16.390	1.310
53	12	S	BS	318	15.700	2.200
52	12	S	Hol/A	250	18.900	2.250
119	12	S	H	250	23.575	1.400
132	12	S	H	250	25.010	1.885
122	12	S	H	250	27.649	1.321
134	12	M	H	216	30.430	1.025
26	18	S	H	425	39.100	2.000
31	20	S	H	421	18.100	1.400
32	22	S	H	455	22.200	2.100
Mean	11.55			267	21.731	1.415

^aData obtained from cattle submitted to Iowa State University Veterinary Clinic from March 2nd, 1957 to May 2nd, 1959.

^bEstimated.

^cS, castrated male; F, female; M, bull.

^dH, Hereford; SH, Shorthorn; A, Angus; Gue, Guernsey; Hol, Holstein; BS, Brown Swiss.

^eBoth adrenals.

Table 2. Adrenal and pituitary weights of 17 apparently normal cattle^a

Animal number	Age ^b (months)	Sex ^c	Breed ^d	Body weight (kg)	Adrenal weight ^e (gms)	Pituitary weight (gms)
115	10	S	H	284	7.220	1.830
111	10	F	H/Hol	247	10.340	1.535
144	10	M	Hol	227	10.905	1.400
143	11	M	Hol	273	14.155	1.360
112	12	S	H	294	9.935	1.735
110	12	S	H	294	12.900	2.085
128	12	S	SH	328	13.715	1.765
125	12	S	Hol	307	16.830	2.010
116	12	S	H	382	18.840	1.707
127	14	F	Hol	346	13.997	2.006
126	14	S	H	388	15.200	1.810
141	15	S	H	464	-	2.255
142	15	S	H	473	-	1.855
124	16	F	SH	334	11.015	2.030
129	18	F	A	444	18.955	1.930
109	18	S	A	382	21.570	2.500
108	18	S	A	354	22.270	2.435
Mean	13.47			342.41	14.523	1.896

^aData obtained from animals submitted for slaughter at Iowa State University from January 20th, 1959 to October 15th, 1959.

^bEstimated.

^cS, castrated male; F, female; M, bull.

^dH, Hereford; Hol, Holstein; SH, Shorthorn; A, Angus.

^eBoth adrenals.

Embedding in new paraffin followed. Adrenals were sectioned at six micron thickness and mounted on albuminized slides.

After removal and trimming, the pituitaries went through the same processing as the adrenals except that only buffered formalin was used as a fixative. In removing the pituitaries care was taken to leave the stalks attached to the pituitaries. The stalks were approximately one centimeter long. The numerous attached blood vessels of the rete mirabile cerebri were removed by using a small sharp scissors and scalpel. With care the dense diaphragm sellae was removed except for a small crescent shaped area posterior to the stalk. That portion of the diaphragm sellae was five to eight millimeters in its anterior-posterior length and was tightly adherent.

Pituitaries from 18 typical mucosal disease cases and 17 normal cattle were weighed before fixation (Tables 1 and 2). To facilitate penetration by the fixative a sharp razor blade was used to make two deep parallel sagittal cuts from the dorsal side of the gland, one cut being on each side of a centerpiece that contained the infundibulum. After 24 hours fixation the necessary trimming was done to facilitate obtaining mid-sagittal sections of the pituitary (Figure 1) following embedding. Pituitary sections were cut at thicknesses of four to six microns and were mounted carefully with the long axis of the pars distalis parallel to the long axis of the glass slide.

Paraffin prepared sections of the adrenal were stained with hematoxylin and eosin, Crossman's modification of Mallory's connective tissue stain (Crossman, 1937), and Gomori's reticulum stain (Armed Forces

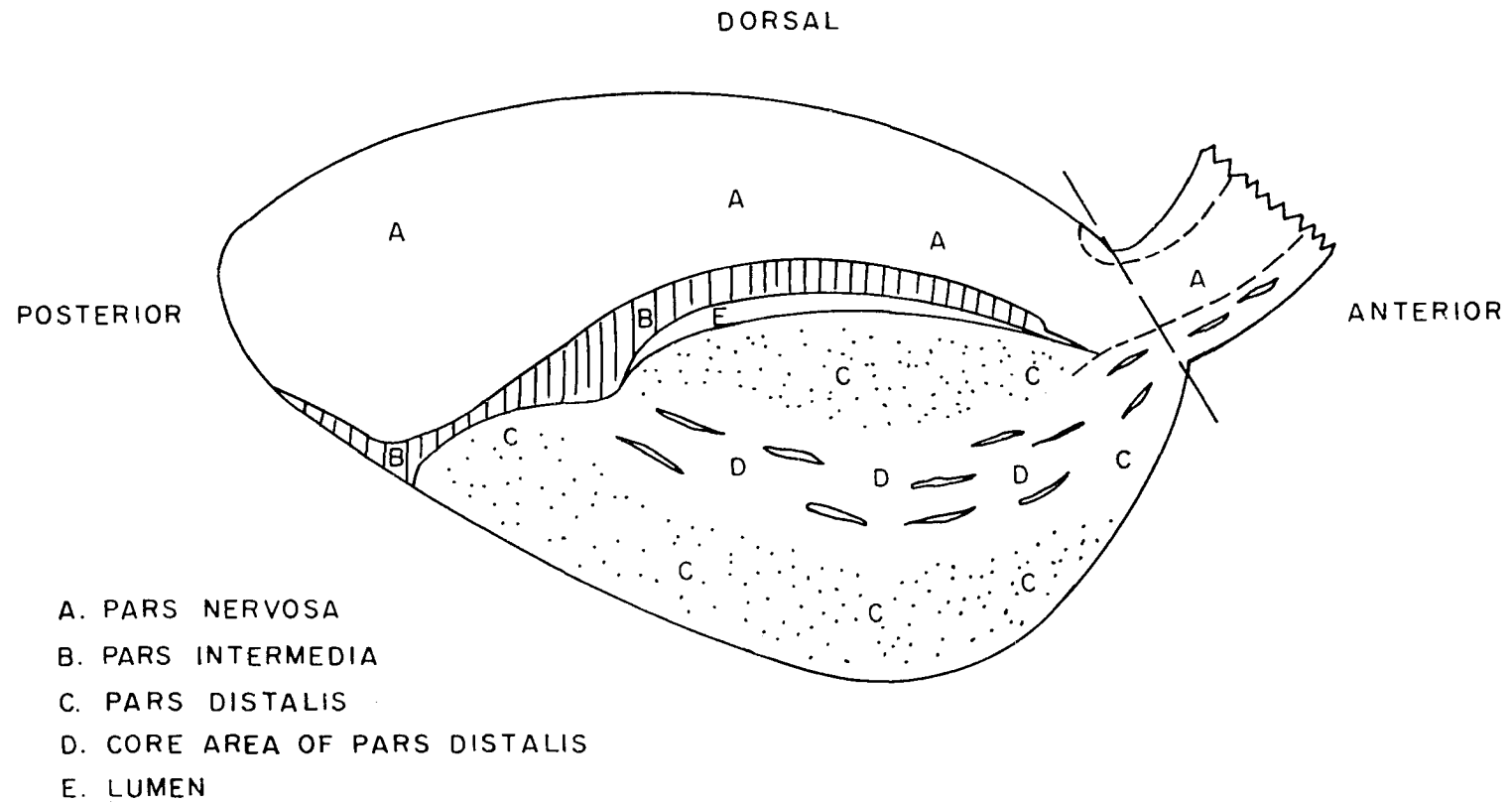


Figure 1. Schematic mid-sagittal
pituitary section

Institute of Pathology, 1957). Paraffin prepared sections of the pituitaries were stained with hematoxylin and eosin, Crossman's modification of Mallory's connective tissue stain, aldehyde fuchsin, as used by Landing (1956), and the periodic acid-Schiff technique as done by Jubb and McEntee (1955b), except that with the latter technique the counterstain was that of Halmi (1952). Control sections were run with the periodic acid-Schiff technique and were always negative.

Frozen sections were cut from calcium-cadmium-formol fixed adrenals at 20 micron and at 30 micron thicknesses. The twenty micron sections were tested in duplicate for the presence of cholesterol using the method described by Weber, Phillips, and Bell (1956). The 30 micron sections were stained with oil red O and counterstained with hematoxylin (Bell, 1959).

As previously related, some adrenals were weighed. In addition, zone measurements were made of two cortical zones of the adrenal, a procedure suggested by the work of Bell (1956), and of Weber, Pritchard, and Sellers (1956). In making the two cortical zone measurements the zona glomerulosa and one half of the zona intermedia were measured together as the first measurement. In normal animals the zona intermedia was distinct, but it was often difficult to detect a distinct zona intermedia in sections from mucosal disease affected adrenals. When the zona intermedia was indistinct, only the zona glomerulosa was included in the first measurement. The combined zona fasciculata-zona reticularis, along with the inner one half of the zona intermedia, was measured as the second measurement. For the sake of convenience in terminology the first

measurement is referred to as the zona glomerulosa measurement in Figure 22 and in the text. The second measurement is referred to as the zona fasciculata measurement in Figure 29 and in the text.

Fifteen evenly spaced circumferential zone measurements were made for each of the two zones described above. Mean thicknesses of each of the two specified zones of each adrenal were plotted separately against the estimated age of the donor animal (Figures 22 and 29). A mean zone measurement and mean age determination was made for both the affected group of cattle and for the normal cattle.

In addition to zone measurements made on paraffin prepared sections of the adrenals, adrenal nuclei were counted in certain unit areas* of the adrenal cortices. The method used was that described by Weber, Pritchard, and Sellers (1956). Nuclei counts were made to determine whether changes in parenchymatous cell size or number were present in affected adrenals. Nuclei counts were made in the midzone of the zona glomerulosa and in the midzone of the combined zona fasciculata-zona reticularis.

To accomplish the adrenal nuclei counting a five millimeter square net micrometer ruled into 25 equal squares was mounted in one ocular. The four corner squares were focused simultaneously on the area to be counted and no adjustment was made in focus until the nuclei in all four corner squares were counted. The counting was performed under 430X magnification. All nuclei were counted that were clearly visible within the four corner squares or that were touching either the top or left hand

*Approximately 525 square microns.

edges of the four squares. Ten circumferentially spaced net micrometer fields were counted in the midzone of the zona glomerulosa. Likewise, ten net micrometer fields were counted in the midzone of the combined zona fasciculata-zona reticularis. A mean count of cell nuclei per unit area was computed for each of the two locations counted. This was done for each animal. Nuclei counts for the zona glomerulosa and for the zona fasciculata-zona reticularis midzone were plotted separately against estimated age of the donor animal (Figures 21 and 30). Total mean nuclei counts and total mean age determinations were then made for the affected cattle and for the apparently normal cattle (Figures 21 and 30).

Differential counts of parenchymatous cells in the pars distalis (Tables 3 and 4) were made on single mid-sagittal pituitary sections (Figure 1). The cells were classified as acidophils, basophils, or chromophobes on the basis of their morphology and the tinctorial properties of their intracytoplasmic granules. All counts were made on sections stained by the periodic acid-Schiff staining procedure as described by Jubb and McEntee (1955b). The third class of parenchymatous cells, commonly believed to be either degranulated pre-secretory or post-secretory acidophils or basophils, exhibited no visible granules in their cytoplasm. They were classified as chromophobes.

To accomplish the cell counting in the pars distalis a five millimeter square net micrometer ruled into 25 equal squares was mounted in an ocular of a binocular microscope. When used with a 40X fluorite oil immersion objective* each square of the net micrometer encompassed

*Bausch and Lomb Optical Co., Rochester, New York.

Table 3. Differential parenchymatous cell counts of the partes distales of 20 mucosal disease affected cattle^a

Case no.	Age ^b (mo)	Sex ^c	Breed ^d	Body weight ^b (kg)	% Acido-phils	% Baso-phils	% Chromo-phobes	Total cells counted
106	6	F	H	182	43.90	12.19	43.90	123
131	8	F	A	182	44.50	14.13	41.36	191
121	8	F	SH	261	35.13	9.72	55.13	185
70	9	S	H	273	64.25	17.87	17.87	207
76	9	S	H/A	277	41.39	0.00	58.60	186
130	10	F	SH/H	182	45.80	2.58	51.61	155
27	10	F	Hol	273	26.38	14.11	59.50	163
66	10	F	BS	273	36.86	10.13	52.99	217
118	11	S	H	250	55.00	1.81	43.18	220
79	11	S	H	318	42.37	0.56	57.06	177
134	12	M	H	216	41.53	9.23	49.23	130
119	12	S	H	250	52.40	0.00	47.59	187
122	12	S	H	250	50.00	3.75	46.25	160
132	12	S	H	250	37.10	10.40	52.48	221
52	12	S	Hol/A	250	58.49	0.00	41.50	253
90	12	S	H	295	51.38	0.00	48.61	181
26	18	S	H	425	31.94	7.40	60.64	216
31	20	S	H	421	41.19	0.37	58.42	267
32	22	S	H	455	26.47	15.68	57.84	204
89	26	S	H	455	44.71	11.05	44.23	208
Mean	12.5			287	43.53	7.05	49.39	

^aData obtained from cattle submitted to Iowa State University veterinary clinic from March 16th, 1956 to May 2nd, 1959.

^bEstimated.

^cF, female; S, castrated male; M, bull.

^dH, Hereford; A, Angus; SH, Shorthorn; Hol, Holstein; BS, Brown Swiss.

Table 4. Differential parenchymatous cell counts of the partes distales of 14 apparently normal cattle^a

Case no.	Age ^b (mo)	Sex ^c	Breed ^d	Body weight (kg)	% Acido-phils	% Baso-phils	% Chromo-phobes	Total cells counted
111	10	F	H/Hol	247	69.10	8.94	21.95	123
115	10	S	H	284	43.35	9.09	47.55	143
110	12	S	H	294	48.35	3.28	48.35	213
112	12	S	H	294	56.57	2.85	40.57	175
125	12	S	Hol	307	49.07	24.07	26.85	216
128	12	S	SH	328	59.60	5.96	34.43	151
116	12	S	H	382	47.46	3.16	49.36	158
127	14	F	Hol	345	54.90	17.15	27.94	204
126	14	S	H	388	39.89	3.53	56.56	198
124	16	F	SH	334	50.98	18.30	30.71	153
108	18	S	A	354	54.21	1.80	43.97	166
109	18	S	A	382	61.30	7.03	31.65	199
129	18	F	A	444	58.76	5.67	35.56	194
113	36	F	H	383	49.30	18.60	32.09	215
Mean	15.2			340	53.06	9.24	37.68	

^aData obtained from cattle submitted for slaughter at Iowa State University between January 20th, 1959 and April 2nd, 1959.

^bEstimated.

^cF, female; S, castrated male.

^dH, Hereford; Hol, Holstein; SH, Shorthorn; A, Angus.

approximately 676 square microns. Two non-adjacent squares of the 25 equal squares were arbitrarily selected as the ones to be used for nuclei counting. In this work the square above and the square below the center square were used in counting.

Under 100X magnification the net micrometer was focused on the cells of the postero-ventral edge of a mid-sagittal section of the pars distalis near the capsule (Figure 1). Oil was applied to the coverslip and a 40X fluorite oil immersion objective was brought to focus without moving the stage. All nuclei that were within the two pre-selected squares, or touching the top or left hand edge of the two squares, were classified and counted. Varying the light intensity and adjusting the fine adjustment screw permitted classification of the various cells throughout the thickness of the tissue being counted.

After the count was completed and recorded for the first net micrometer field, the 10X objective was again focused and the stage moved so that the field of view progressed anteriorly and along the capsule for five successive adjacent net micrometer fields. The fifth field, lying just within the capsule, was counted under oil after the fluorite lens had been substituted for the 10X objective. After counting and recording the nuclei, the 10X objective was used again to measure off three successive adjacent net micrometer fields, but this time in a direction transverse to the long axis of the pars distalis, thus progressing ventro-dorsally. The third field was counted at 400X magnification under oil. In the same manner every third net micrometer field, as measured under 100X magnification, was counted under oil at 400X magnifi-

cation.

Eventually the lumen of the adenohypophysis, or the border between the pars intermedia and the pars distalis was reached. Then the stage was moved again, under 100X magnification, so as to advance the field of view anteriorly for another five successive adjacent net micrometer fields. The fifth field, lying next to the lumen or next to the pars intermedia-pars distalis border, was counted under oil at 400X magnification. After returning to 100X magnification, the direction of movement was shifted so as to cross the long axis of the pars distalis in a dorso-ventral direction. Again every third net micrometer field, as measured under 100X magnification, was counted under oil at 400X magnification.

In the manner just described the pars distalis was repeatedly traversed under 100X magnification and every third net micrometer field was counted under 400X magnification after substituting the fluorite oil immersion objective for the 10X objective. When necessary, the stage was moved so as to shift the field of view anteriorly for five adjacent net micrometer fields. The pars tuberalis was not included in the counts.

This method resulted in the counting of 10 to 21 net micrometer fields per pars distalis. The number of fields counted varied with the size of the gland. The mean number of net micrometer fields counted was 15.7 fields per section. The total number of cells counted varied from 127 to 267 cells per pars distalis.

FINDINGS

Gross Observations

The adenohypophyses

The adenohypophyses from cattle with mucosal disease appeared normal on gross observation. Figure 2 graphically presents data on the pituitary weights of 18 mucosal disease affected cattle and 17 normal cattle. The pituitary weights were plotted against the estimated ages of the cattle. The mean pituitary weight of 18 mucosal disease affected cattle was 1.41 grams and the mean age of the donors was 11.55 months. The mean pituitary weight for 17 normal cattle was 1.89 grams and the mean age of the donor group was 13.47 months. Tables 1 and 2 present, in tabular form, the same information plotted in Figure 2.

The adrenals

The adrenals of mucosal disease affected cattle were usually enlarged and this was reflected in increased weights (Tables 1 and 2). Figure 3 graphically presents data on total adrenal weights of 18 mucosal disease affected cattle and of 15 normal cattle. The adrenal weights were plotted against the estimated ages of the cattle. The mean adrenal weight of the affected group of cattle was 21.73 grams and the mean age of the same group was 11.55 months. The mean adrenal weight of 15 normal cattle was 14.52 grams and the mean age of the same group was 13.26 months. Tables 1 and 2 include the same data plotted in Figure 3, but in tabular form.

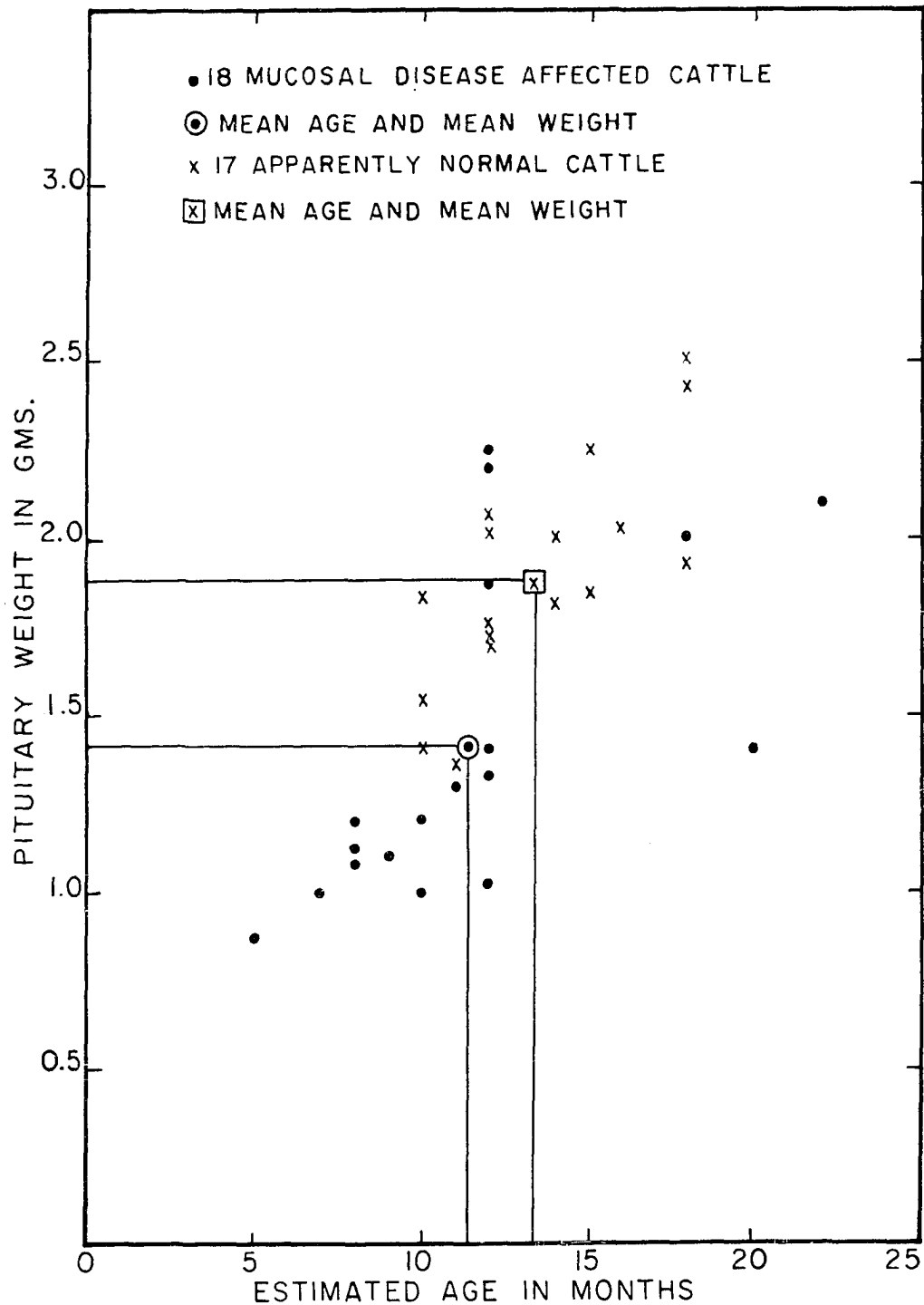


Figure 2. Pituitary weights plotted against estimated ages of cattle

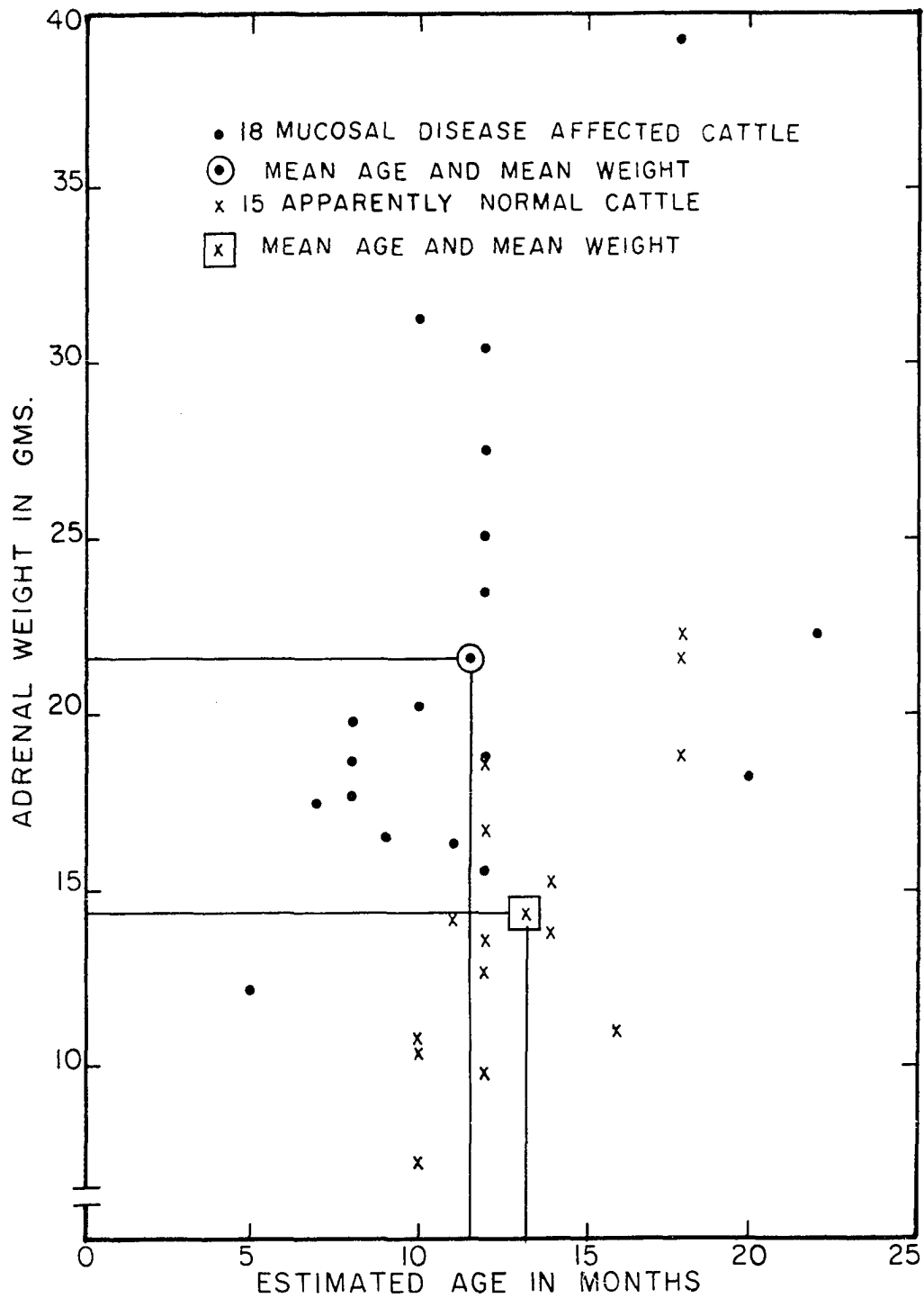


Figure 3. Total adrenal weights plotted against estimated ages of cattle

No consistent color difference was observed between the adrenals of affected cattle and normal cattle. There was considerable variation in the color of the adrenals from both groups of cattle. Adrenal color varied from a tan to a light yellow. The incised surfaces of enlarged adrenals bulged. Occasionally an affected animal yielded adrenals that were soft and greasy on cut surface.

Cattle that were allowed to die in the usual course of mucosal disease, or that were destroyed when moribund, presented adrenals in which variable numbers of petechial and ecchymotic hemorrhages were visible through the capsule. Cattle that received euthanasia before they became moribund failed to present hemorrhages in the adrenal.

Microscopic Observations

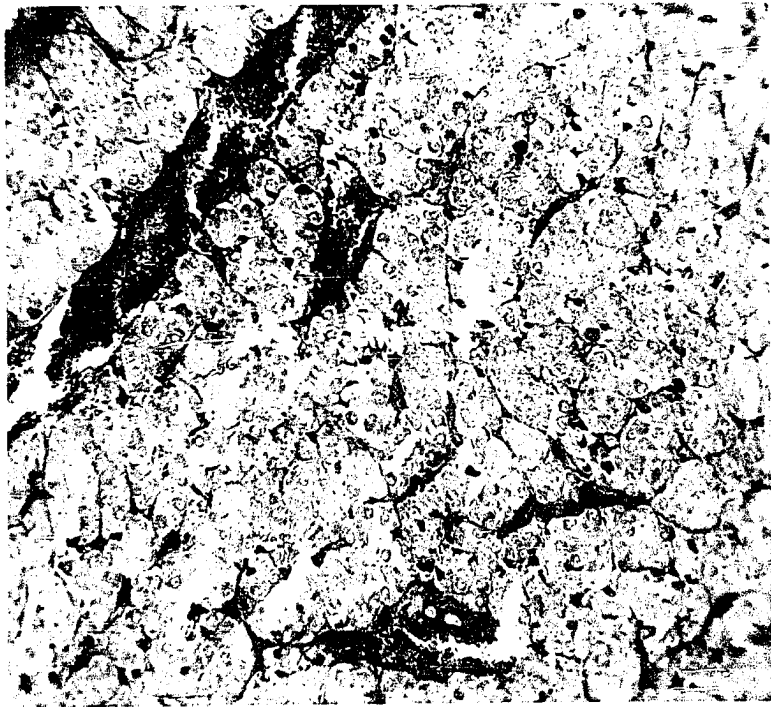
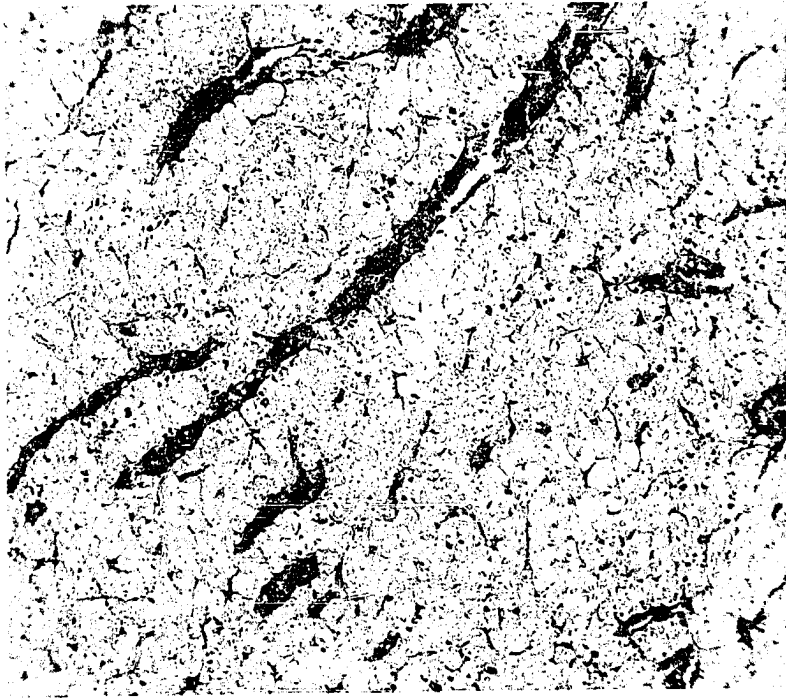
The adenohypophyses

In the adenohypophyses of mucosal disease affected cattle a marked congestion was present in the large sinusoids of the core area and in the small capillaries between the acini (Figures 4 and 5). There was no indication of inflammatory change.

Differential cell counts were made of the parenchymatous cells of the partes distales of 20 affected cattle and 14 normal cattle (Tables 3 and 4). The percentual relationship of the various parenchymatous cells was determined and given. The cells were classified as acidophils, basophils, and chromophobes. On the basis of total mean counts for the three classes of parenchymatous cells there were less acidophils, less basophils, and more chromophobes in the partes distales of mucosal

Figure 4. Congestion of the large sinusoids and small capillaries in the core area of the pars distalis. X 96. From a heifer with mucosal disease. A periodic acid - Schiff stain with a Halmi counterstain

Figure 5. An enlargement of a portion of the field shown in Figure 4. X 190



disease affected cattle. Figure 6 graphically illustrates the overall mean percentual relationship of the parenchymatous cells found in 20 mucosal disease affected cattle and in 14 normal cattle. The same information is given in tabular form in Tables 3 and 4. Figure 6 shows that the overall mean cell percentages in 20 affected cattle were 43.5 percent acidophils, 7.0 percent basophils, and 49.4 percent chromophobes. The overall mean cell percentages of 14 normal cattle were shown to be 53.0 percent acidophils, 9.2 percent basophils, and 37.7 percent chromophobes.

Upon close observation of the partes distales of normal and affected cattle a diffuse, partial to complete loss of intracytoplasmic granules was observed in the acidophils of affected cattle (Figures 7 and 8). Furthermore, within those acini that were composed predominantly of acidophils, increased numbers of completely degranulated acidophils were observed (Figures 9 and 10). Those degranulated acidophils were classified as chromophobes, as were all completely degranulated parenchymatous cells. Basophils were similar in sections from normal animals and mucosal disease affected animals.

Adrenal cortices

The capsules of adrenals from mucosal disease affected cattle varied in thickness, but measurements of this variation were not made. Adrenals that were markedly increased in size and weight frequently were observed to have capsules that were reduced in thickness (Figures 11 and 12). However, exceptions were noted. In most instances reduced capsule thickness

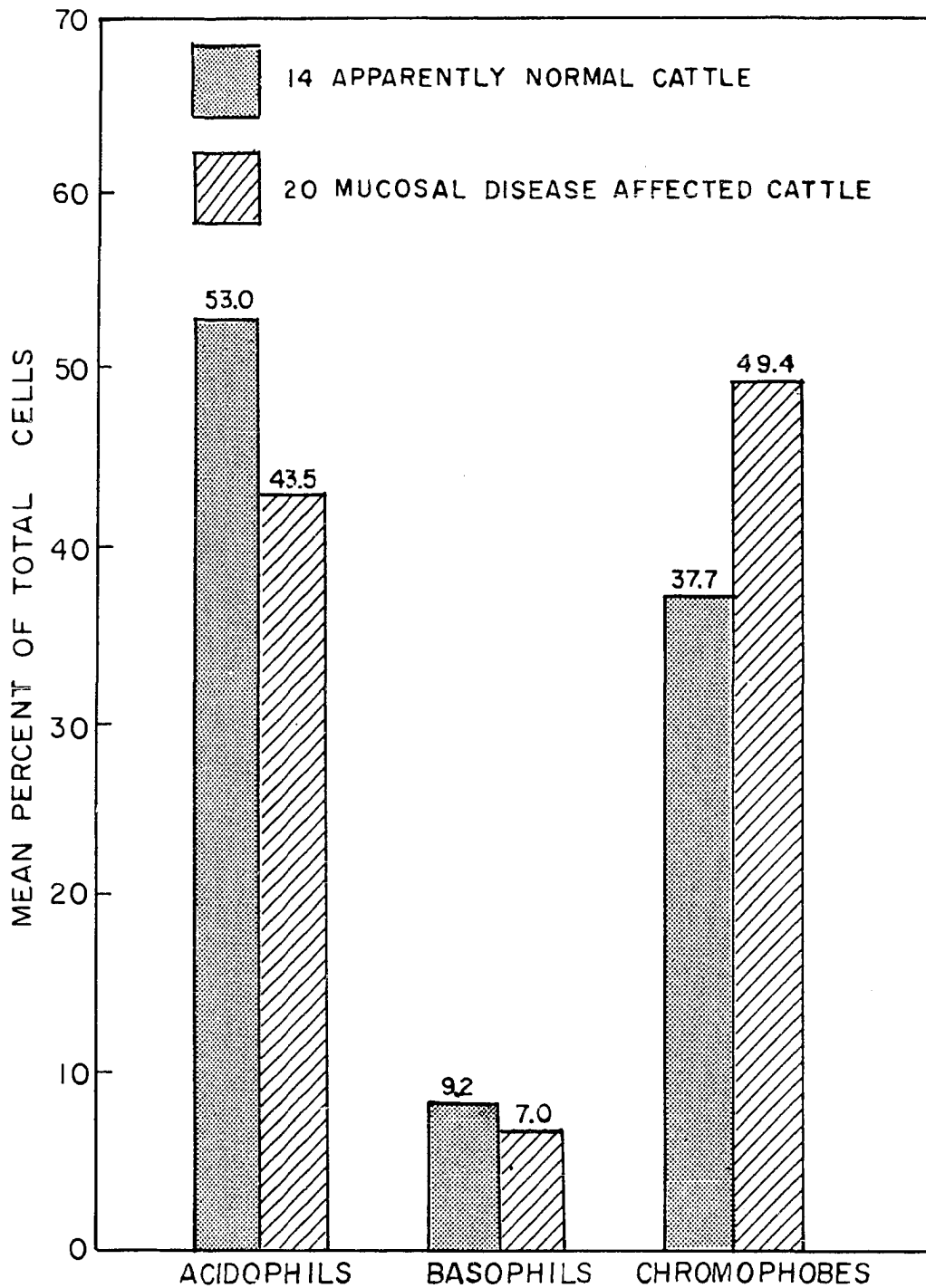


Figure 6. Comparison of the parenchymatous cells in the adenohypophyses of mucosal disease affected cattle and apparently normal cattle

Figure 7. Acidophils in the adenohypophysis of a normal steer. X 486.
Note the numerous intracytoplasmic granules. A periodic acid -
Schiff stain with a Halmi counterstain

Figure 8. Partially degranulated and completely degranulated acidophils
in the adenohypophysis of a mucosal disease affected steer.
X 486. Note the marked loss of intracytoplasmic granules
from the acidophils. A periodic acid - Schiff stain with a
Halmi counterstain

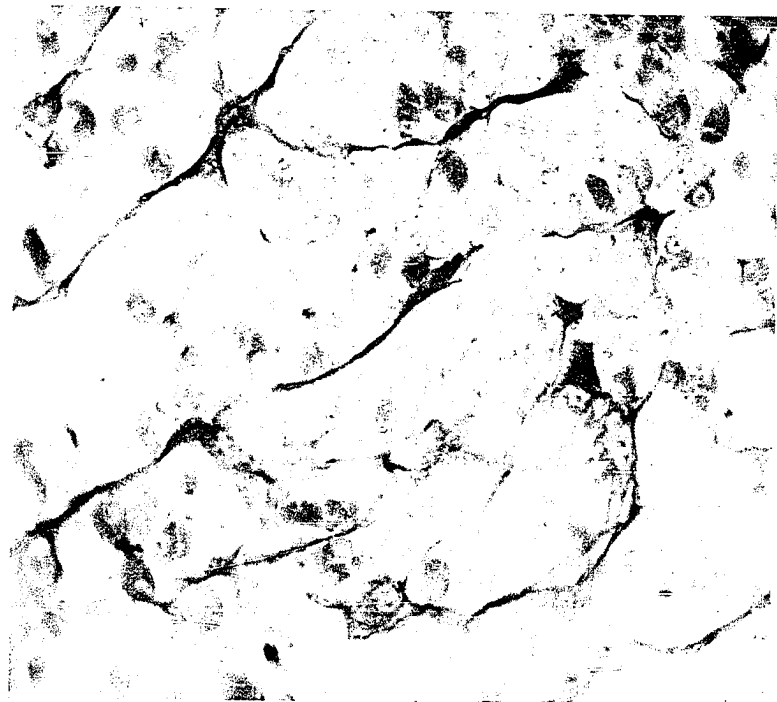
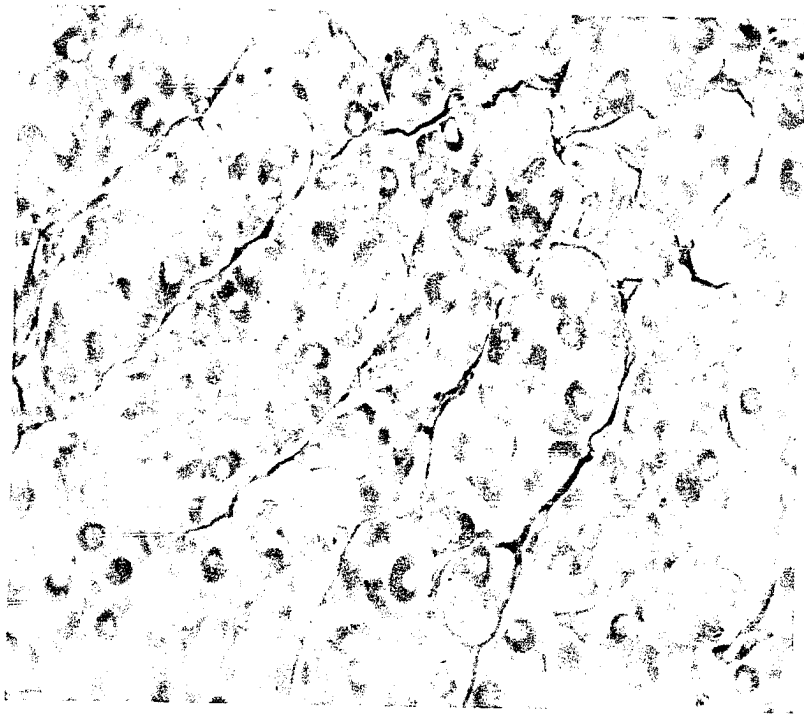


Figure 9. Acini, composed predominantly of acidophils, in the adenohypophysis of a normal steer. X 885. Note the numerous acidophilic granules in the acidophils.
A periodic acid - Schiff stain with a Halmi counterstain

Figure 10. Degranulated acidophils in acini that were composed predominantly of acidophils before degranulation occurred. From a mucosal disease affected steer. X 885. A periodic acid - Schiff stain with a Halmi counterstain

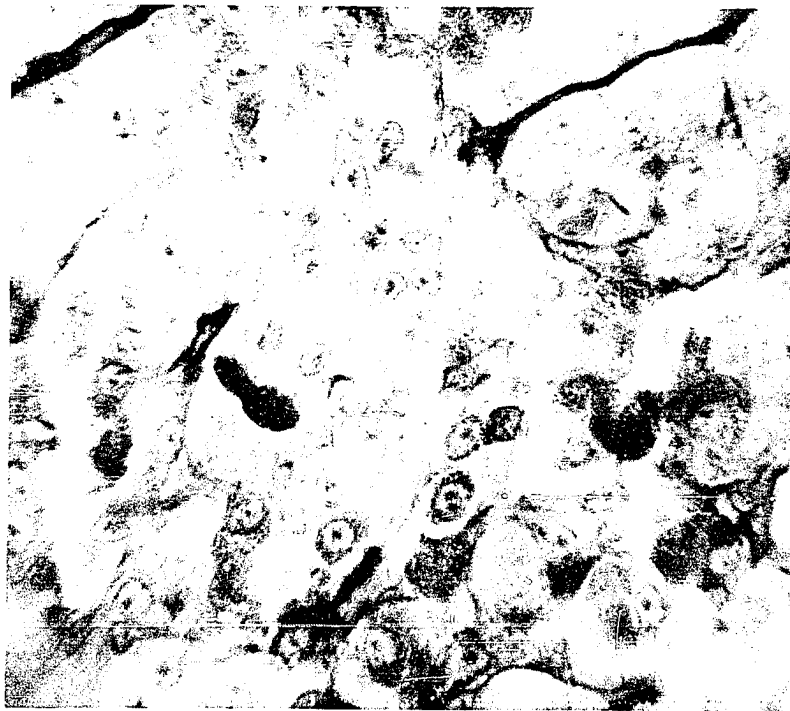
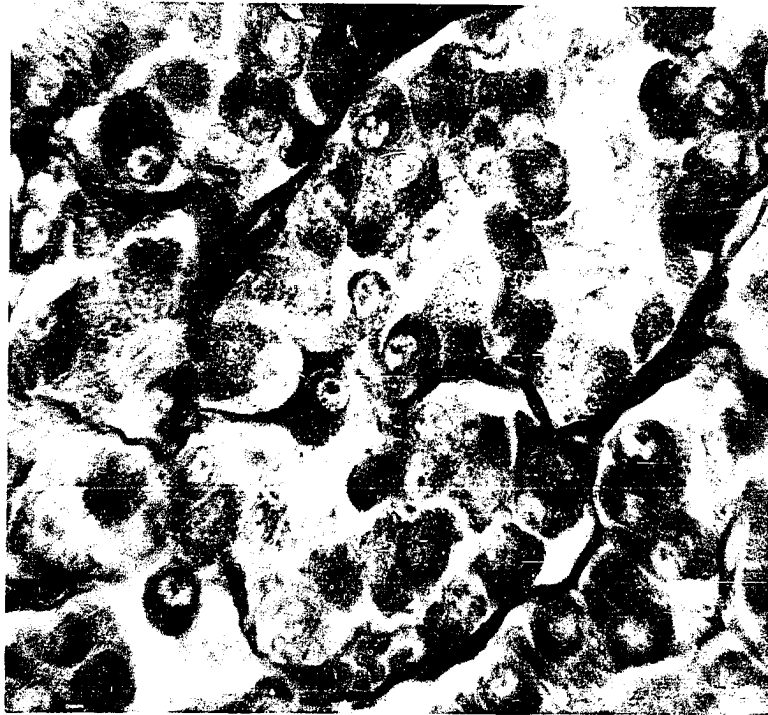
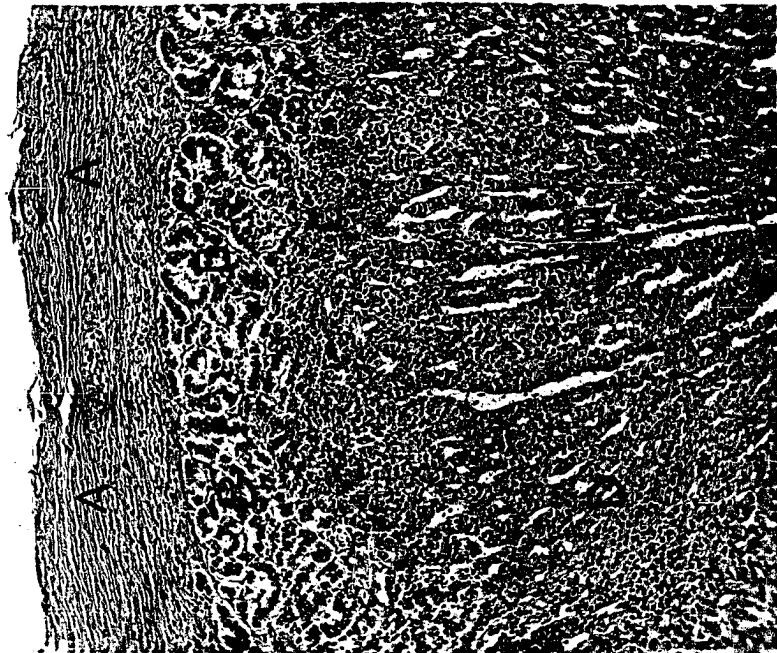
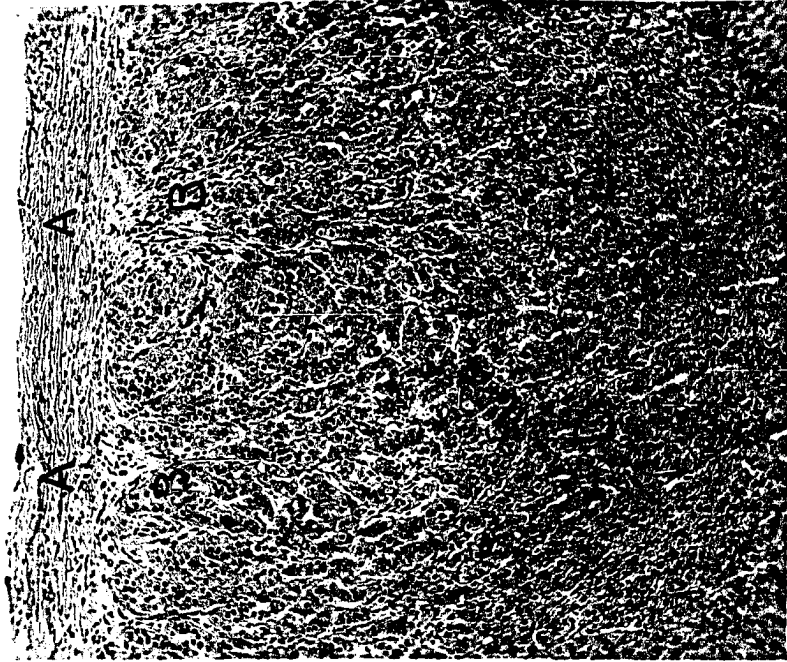


Figure 11. A section from a normal bovine adrenal cortex. X 96. Note the thick capsule, the loose structure of the zona glomerulosa, the distinct zona intermedia, and the numerous sinusoids in the zona fasciculata. A, capsule; B, zona glomerulosa; C, zona intermedia; D, zona fasciculata

Figure 12. A section from the adrenal cortex of a mucosal disease affected bull. X 96. Note the thin capsule, the fully-packed structure of the zona glomerulosa, the absence of a distinct zona intermedia, and the absence of visible sinusoids in the zona fasciculata. Compare with Figure 11. A, capsule; B, zona glomerulosa; D, zona fasciculata



appeared to be due to a decrease in the thickness of the inner, cellular part of the capsule. Adrenals that were not markedly increased in size and weight had capsules of the thickness noted in control animals.

In the adrenals of all of 19 cattle that died of mucosal disease a lymphocytic infiltration always was present where the inner cellular part of the adrenal capsule adjoined large trabeculae supporting afferent blood vessels (Figures 13 and 14). Lymphocytic infiltrations sometimes extended along the trabeculae for some distance toward the medulla (Figures 15 and 16). Occasional plasma cells sometimes were observed among the lymphocytes. It was often necessary to examine more than one trabecula-capsule junction to detect the cellular infiltration. However, a single transverse adrenal section always sufficed to demonstrate the lymphocytic infiltration. Adrenals from 18 of 23 affected cattle that were destroyed also exhibited the same cellular infiltration, whereas five animals failed to do so.

Lymphocytic infiltrations, as just described, varied in intensity. The number of lymphocytes present in an adrenal trabecula-capsule junction, as seen in a six micron thick section, varied from approximately six to 150 cells. In adrenals in which the lymphocytic infiltration was marked, occasional scattered lymphocytes were observed also along the inner cellular part of the entire capsule. In adrenals exhibiting only mild infiltrations of lymphocytes the infiltrating cells were confined to trabeculae-capsule junctions. Lymphocytes seldom were observed throughout the various zones of the cortical parenchyma or within the sinusoids.

Figure 13. Lymphocytes in the trabecula-capsule junctions in an adrenal from a mucosal disease affected steer. X 96. A, capsule; B, lymphocytes; C, nerve. Crossman's modification of Mallory's connective tissue stain

Figure 14. Lymphocytes in a trabecula-capsule junction in the adrenal of a mucosal disease affected steer. X 486. Crossman's modification of Mallory's connective tissue stain

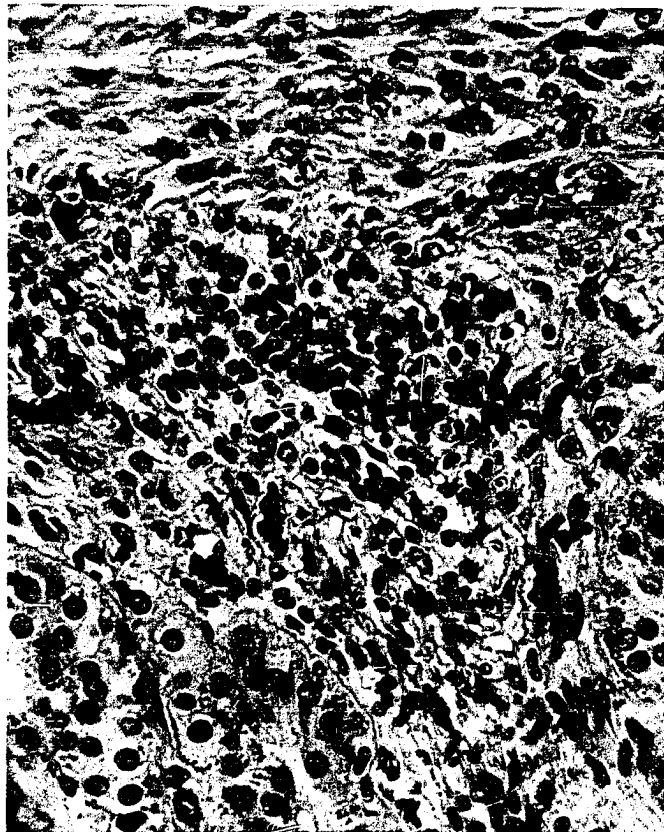
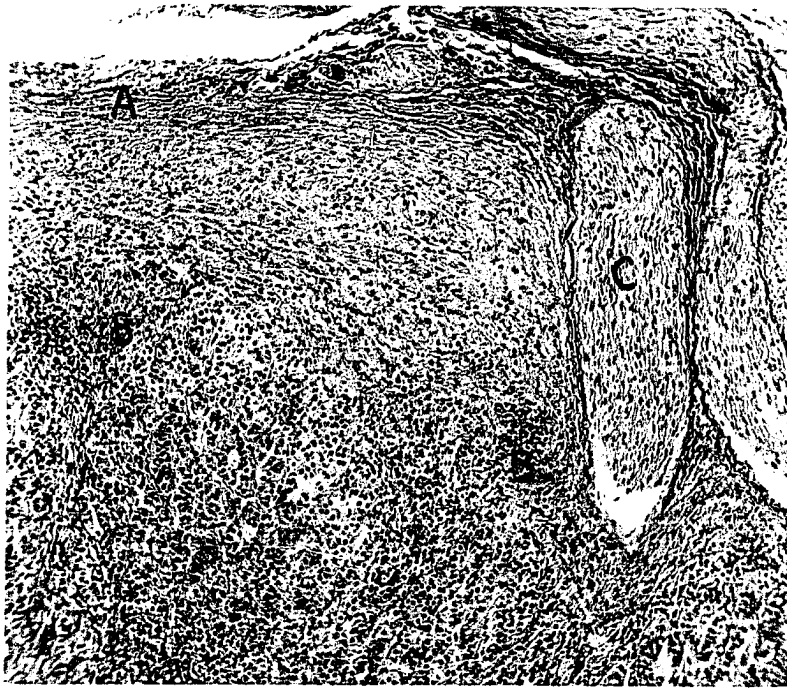
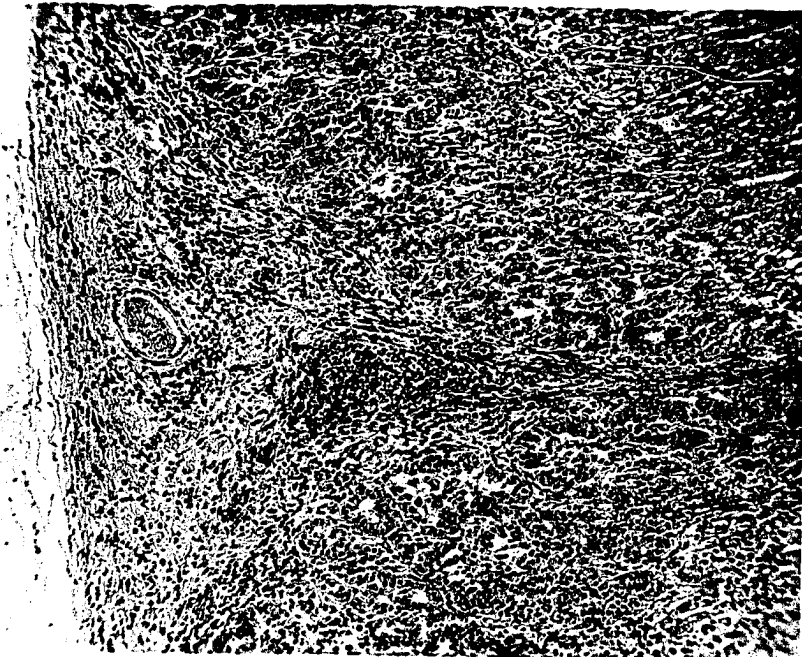
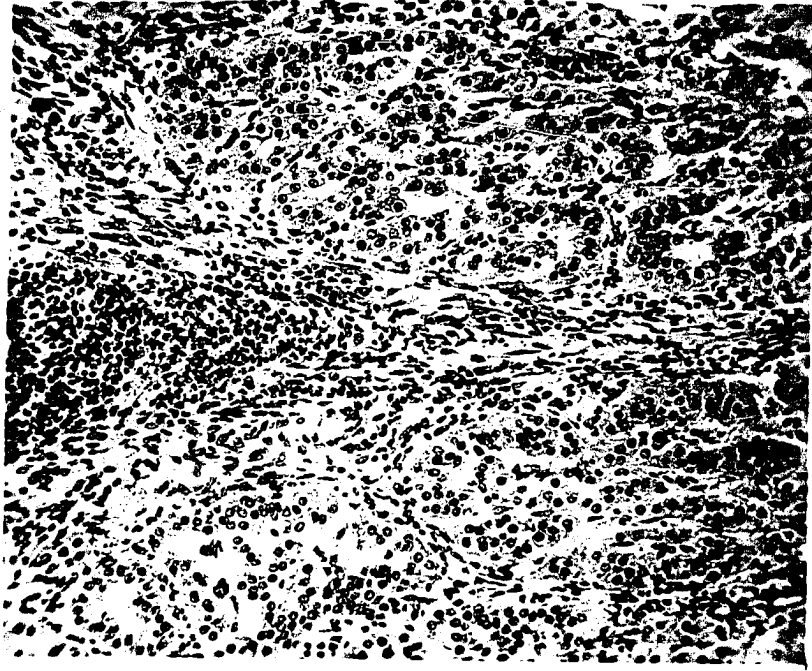


Figure 15. Lymphocytic infiltration extending along a trabecula in the adrenal of a mucosal disease affected steer. X 96

Figure 16. An enlarged portion of the field shown in Figure 15. X 190



Eosinophils occasionally were found, and neutrophils rarely were found, in the accumulations of lymphocytes just described (Figures 17 and 18). Although the granulocytes were never as numerous as the lymphocytes, from two to ten were commonly counted among the lymphocytes in a trabecula-capsule junction. Scattered individual eosinophils and neutrophils were observed throughout all cortical zones and were especially noticeable in sinusoids of adrenals containing cortical hemorrhage or marked cell necrosis.

In sections from normal bovine adrenals the normal loose glomerular structure of the bovine zona glomerulosa was readily observed (Figures 11 and 19). The hollow, collapsed-ball-like structure of the zona glomerulosa (Figure 19), as described by Elias (1945), was observed frequently. Adrenals from mucosal disease affected cattle never showed the typical loose zona glomerulosa structure, and the collapsed-ball-like arrangement of the zona glomerulosa cells was seldom observed. Instead, the zona glomerulosa was composed of closely packed, enlarged parenchymatous cells (Figures 12, 20 and 21). Compare Figure 20 with Figure 19. Zona glomerulosa parenchymatous cells occasionally exhibited cloudy swelling or minor fatty alterative changes, but usually had normal appearing cytoplasm.

Two of three cattle, destroyed in the comparatively early stages of mucosal disease, had zona glomerulosa cells similar in size to the cells of normal cattle. Those two adrenal nuclei counts appear near the top of the chart in Figure 21. The width of the zona glomerulosa was increased in most affected adrenals (Figure 22). Cells in the periphery of the

Figure 17. Eosinophils in an infiltration of lymphocytes in an adrenal trabecula-capsule junction. X 486. Section from a mucosal disease affected steer. Crossman's modification of Mallory's connective tissue stain

Figure 18. An enlargement of eosinophils shown in Figure 17. X 885

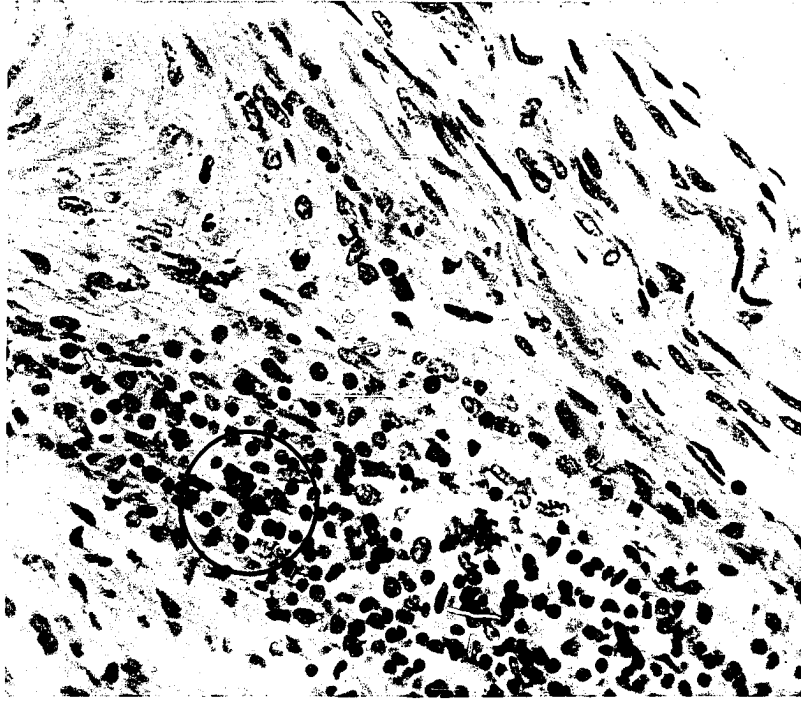


Figure 19. A section from the adrenal cortex of a normal heifer. X 190. Note the hollow, crushed-ball-like arrangement of the zona glomerulosa. A, capsule; B, zona glomerulosa; C, zona intermedia

Figure 20. An adrenal cortical section from a mucosal disease affected heifer. X 190. Note the closely packed structure of the zona glomerulosa and the enlarged cells. Compare with Figure 19. The zona intermedia is indistinct. A, capsule; B, zona glomerulosa

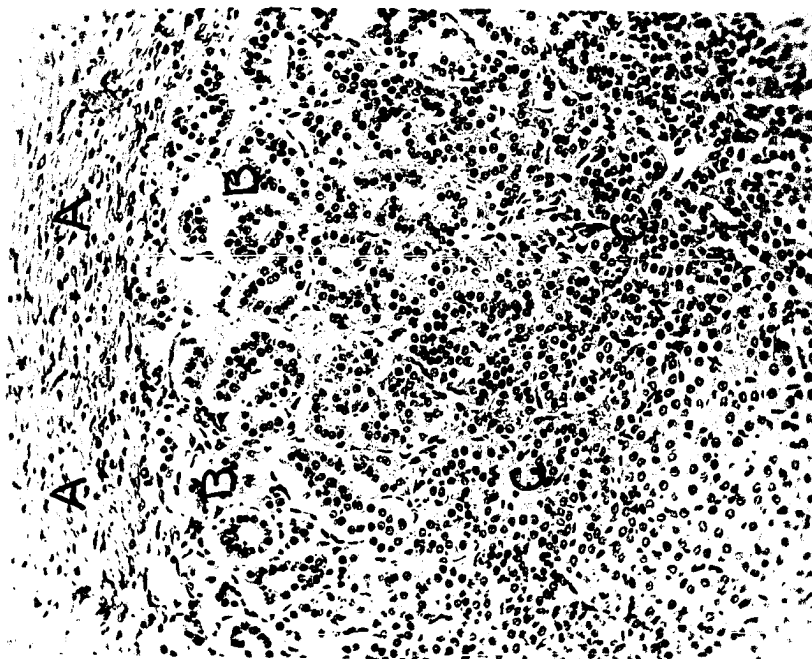
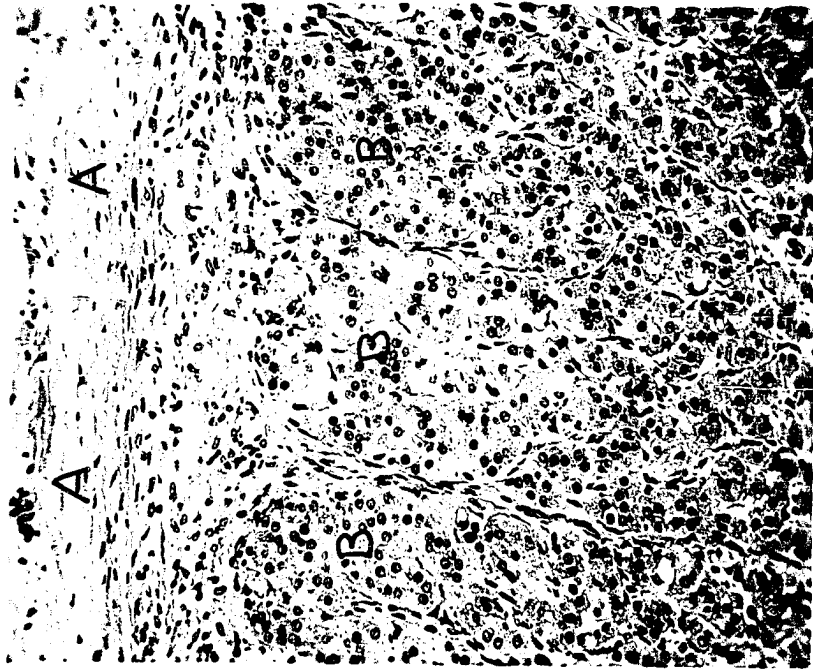


Figure 21. Zona glomerulosa nuclei counts plotted against the estimated ages of cattle. A reduced nuclei count indicates enlargement of the zona glomerulosa cells

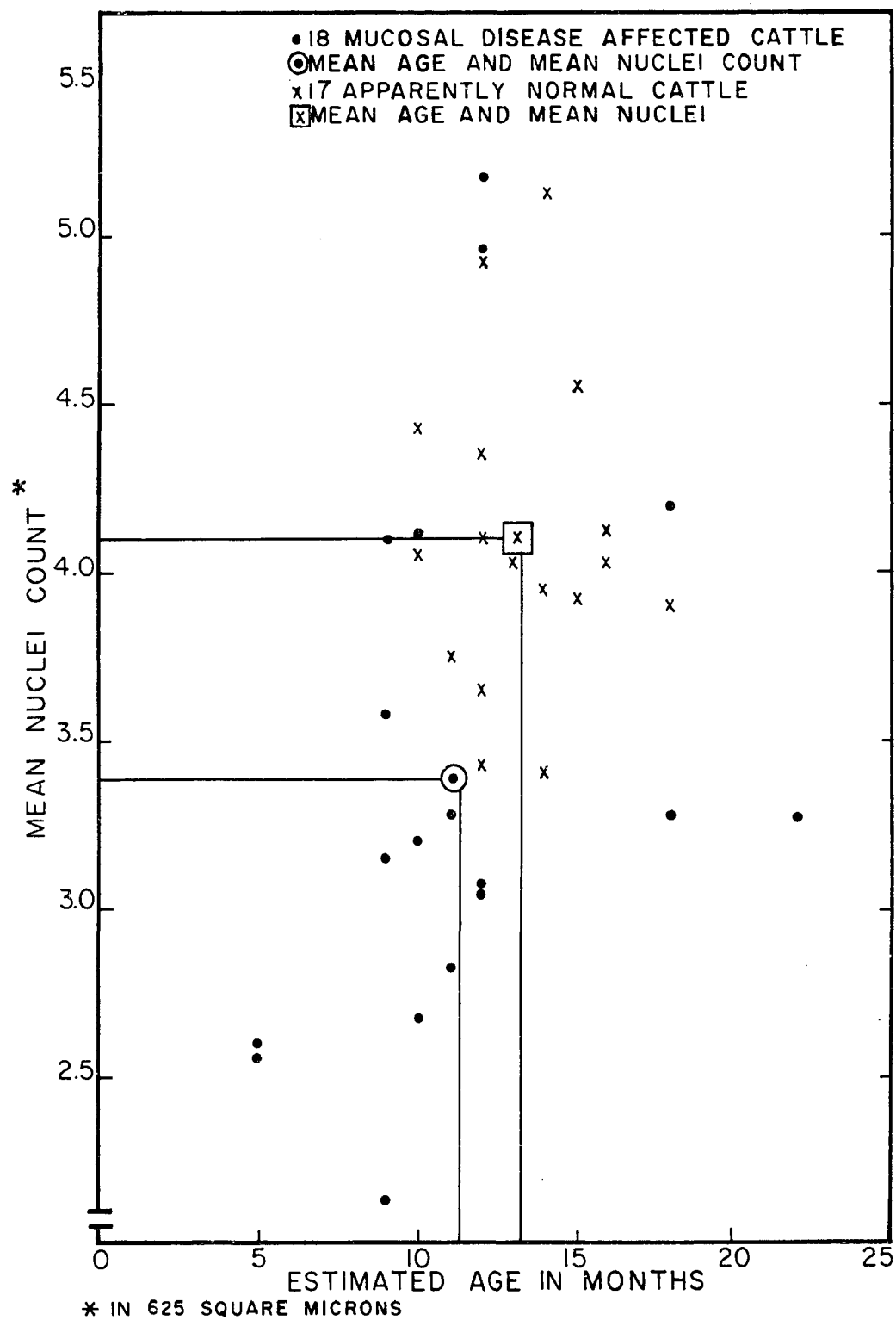
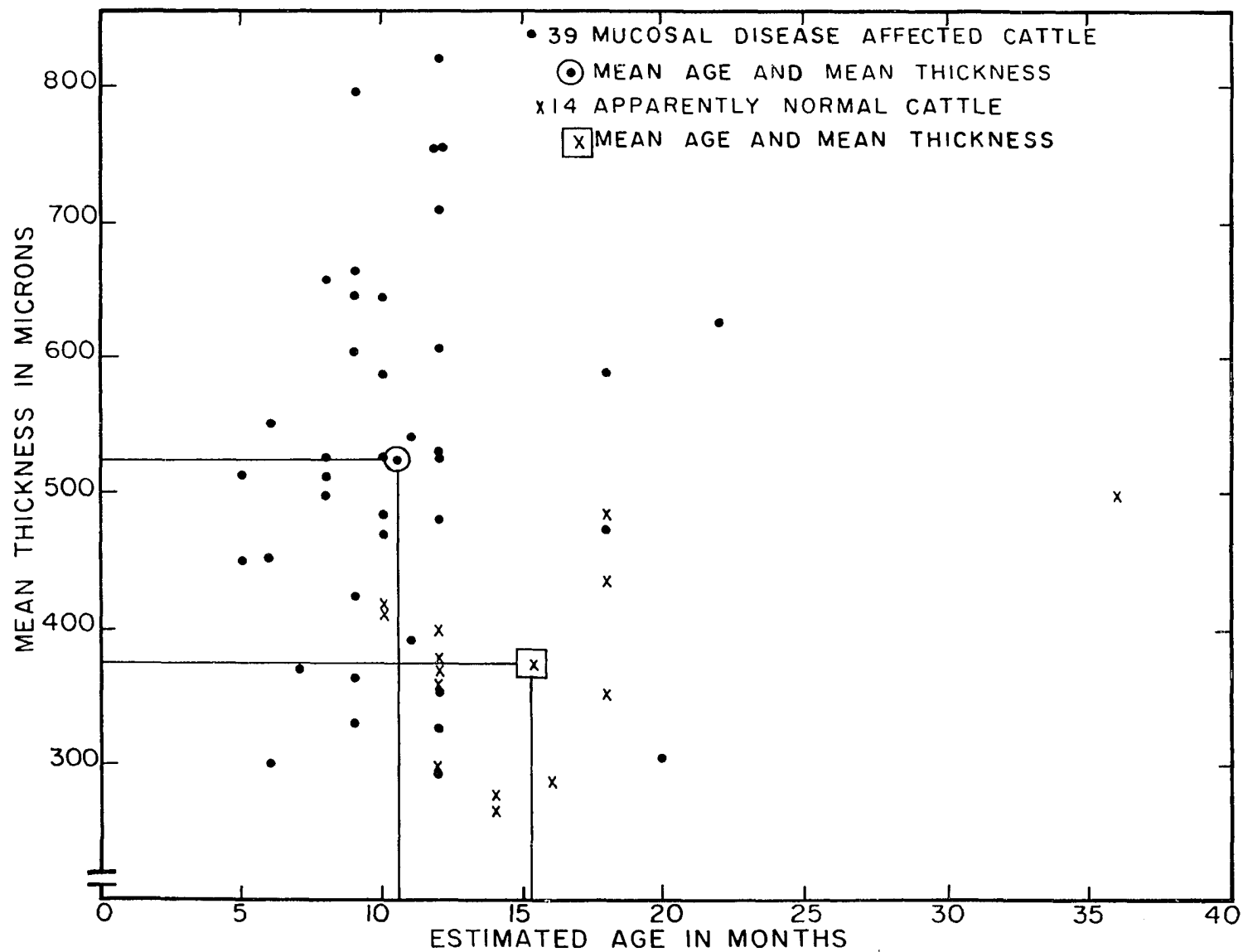


Figure 22. Mean thicknesses of the zonae glomerulosae plotted against the estimated ages of the cattle. Cattle with mucosal disease frequently had thicker zonae glomerulosae than did normal cattle



zona glomerulosa often failed to take the usual amounts of eosin in hematoxylin and eosin stained preparations.

The differences just described in the zonae glomerulosae of affected cattle and normal cattle are reflected in the data given in Figures 21 and 22. Figure 22 presents data on the mean zona glomerulosa thickness in the adrenals of 39 mucosal disease affected cattle and 14 normal cattle. The overall mean zona glomerulosa thickness of 39 affected adrenals was 523 microns and the mean age of the donor cattle was 10.56 months. The mean thickness of the zona glomerulosa of the adrenals of 14 normal cattle was 376 microns and the mean age of the donor group was 15.28 months.

Figure 21 presents data on the mean zona glomerulosa nuclei counts made on the adrenals of 18 mucosal disease affected cattle and 17 normal cattle. The overall mean zona glomerulosa nuclei count for the adrenals of 18 mucosal disease affected cattle was 3.39 nuclei per unit area* and the mean age for the group of affected cattle was 11.3 months. The overall mean zona glomerulosa nuclei count for the adrenals of 17 normal cattle was 4.1 nuclei per unit area and the mean age of the group of normal cattle was 13.3 months.

Weber, McNutt and Morgan (1950) described globular and rod-shaped granules in the cytoplasm of zona glomerulosa cells (Figure 23). They also observed that adjacent cells in the zona fasciculata occasionally had such intracytoplasmic granules. Those observations were confirmed

*Unit area was 525 square microns.

in this study. There appeared to be no difference in the granules in sections from mucosal disease affected cattle and normal cattle. The granules were present in most of the adrenals studied.

Although the zona intermedia is not a well defined zone in the bovine adrenal, it was observed in the adrenals of the normal animals (Figures 11 and 19). In approximately one half of the mucosal disease affected adrenals the zona intermedia was not visibly delineated from the adjacent zona fasciculata, but appeared to be a part of it (Figures 12 and 20).

Petechial and ecchymotic hemorrhages in the affected adrenals were located predominantly within the peripheral one third of the zona fasciculata although they often extended out to the inner zona glomerulosa border (Figure 24). Hemorrhage was seldom observed entirely within the zona glomerulosa. This was surprising in view of the fact that the hemorrhages were seen grossly and were assumed to be just below the capsule.

Hyaline-like droplets (Figures 25 and 26), varying in size from one to 20 microns, were found in the sinusoids of approximately one half of the mucosal disease affected adrenals. Small droplets were observed also in the inner cellular part of the capsule, especially in adrenals from animals that died of mucosal disease. Under 190X magnification, or greater magnification, the larger hyaline-like droplets appeared to be vacuolated. Although hyaline-like droplets were observed in all cortical zones, they were more numerous and larger in sinusoids of the zona reticularis (Figure 25) and in dilated sinusoids between the zona

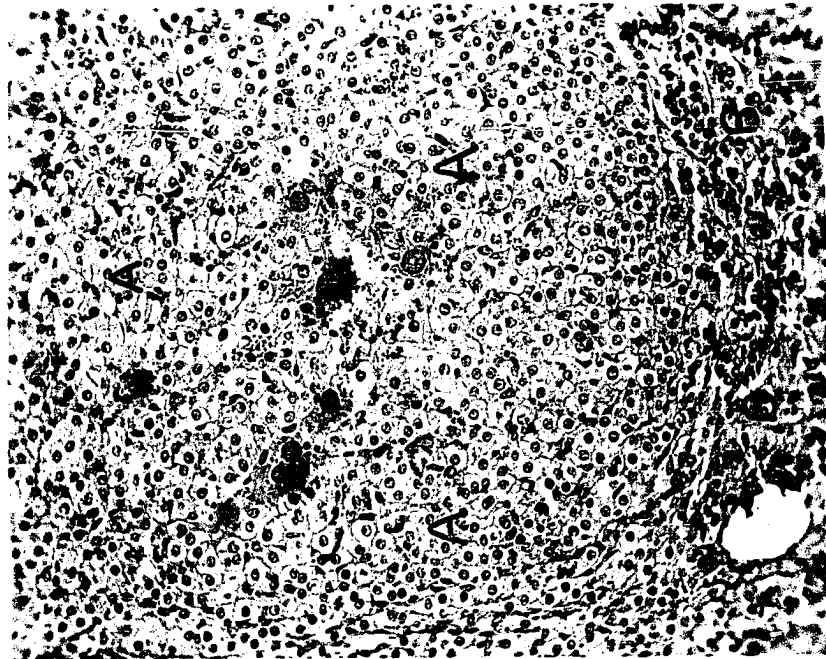
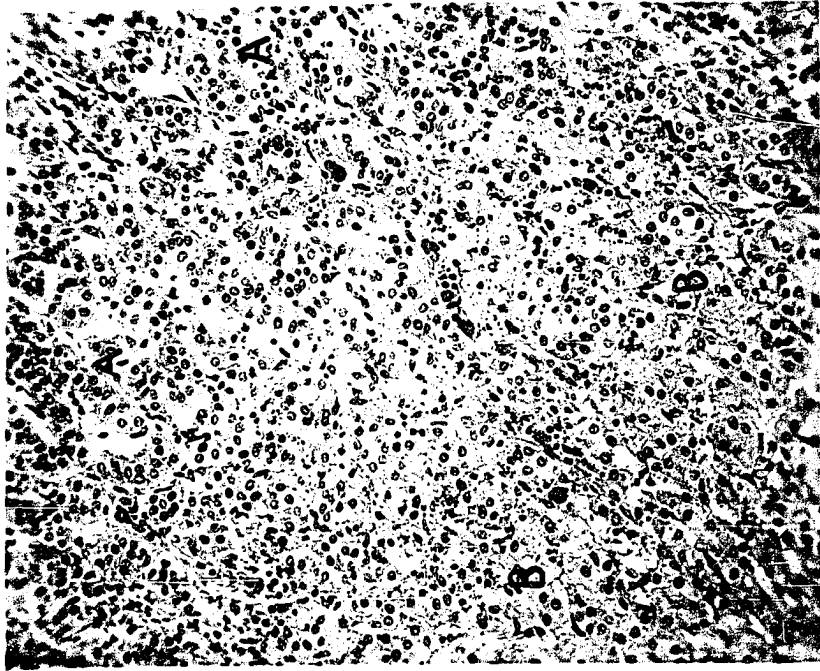
Figure 23. Globular and rod-shaped granules in the cytoplasm of zona glomerulosa cells. From a mucosal disease affected bull. X 486. Crossman's modification of Mallory's connective tissue stain

Figure 24. A petechial hemorrhage present in the peripheral zona fasciculata and extending to the zona glomerulosa. X 486. A, zona fasciculata; B, zona glomerulosa. Crossman's modification of Mallory's connective tissue stain



Figure 25. Hyaline-like droplets in the zona reticularis of a mucosal disease affected adrenal. X 190. A, zona reticularis; B, medulla

Figure 26. Hyaline-like droplets in the sinusoids between the ⁸zona glomerulosa and zona fasciculata. X 190. A, zona glomerulosa; B, zona fasciculata



glomerulosa and zona fasciculata (Figure 26). The droplets were both eosinophilic and fuchsinophilic, and they were more readily detected when stained with fuchsin. Adrenals from cattle that died of mucosal disease contained hyaline-like droplets more often than did the adrenals from cattle that received euthanasia.

The zona fasciculata presented marked fatty alterative changes (Figures 27 and 28) in most mucosal disease affected adrenals. This was especially true of the peripheral one third to one half of the zone. The parenchymatous cells were enlarged and vacuoles of various sizes were present in the cytoplasm. Under 486X magnification the vacuoles varied in size from those just visible to those that displaced the nucleus and filled approximately one half of the cytoplasmic area. Numerous small vacuoles gave the cytoplasm of involved cells a foamy appearance.

The enlargement of parenchymatous cells that occurred in the zona fasciculata influenced two of the measurements made on the adrenal cortices. In affected adrenals the mean width of the zona fasciculata was increased (Figure 29), and the mean nuclei count made in the midzone of the zona fasciculata was decreased (Figure 30).

Figure 29 presents data on the mean zona fasciculata thickness in the adrenals of 39 mucosal disease affected cattle and 14 normal cattle. The thickness of the zona fasciculata was plotted against the estimated age of the cattle in months. The mean zona fasciculata thickness of the 39 affected adrenals was 1893 microns and the mean age of the donor cattle was 10.56 months. The mean thickness of the zona fasciculata of the 14 normal cattle was 1568 microns and the mean age of the same cattle

Figure 27. Fatty alterative changes in the zona fasciculata-zona reticularis. X 190. Section from the adrenal of a mucosal disease affected heifer

Figure 28. Fatty alterative changes in the zona fasciculata of a steer. X 486. Note the foamy appearance of the cytoplasm and the numerous small vacuoles

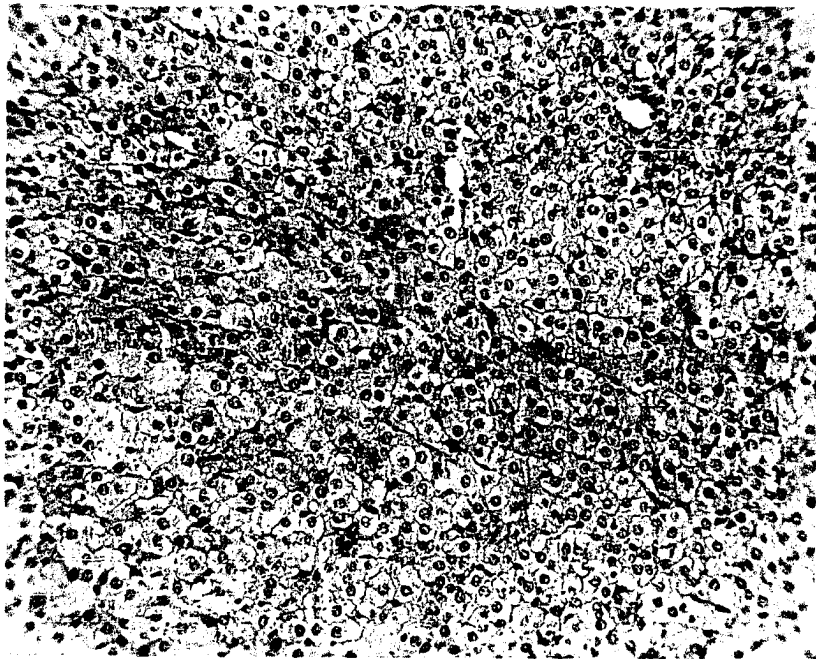


Figure 29. Zona fasciculata thicknesses plotted against estimated ages of cattle. Best use can be made of the data by studying the plottings of the 10-12 month old animals

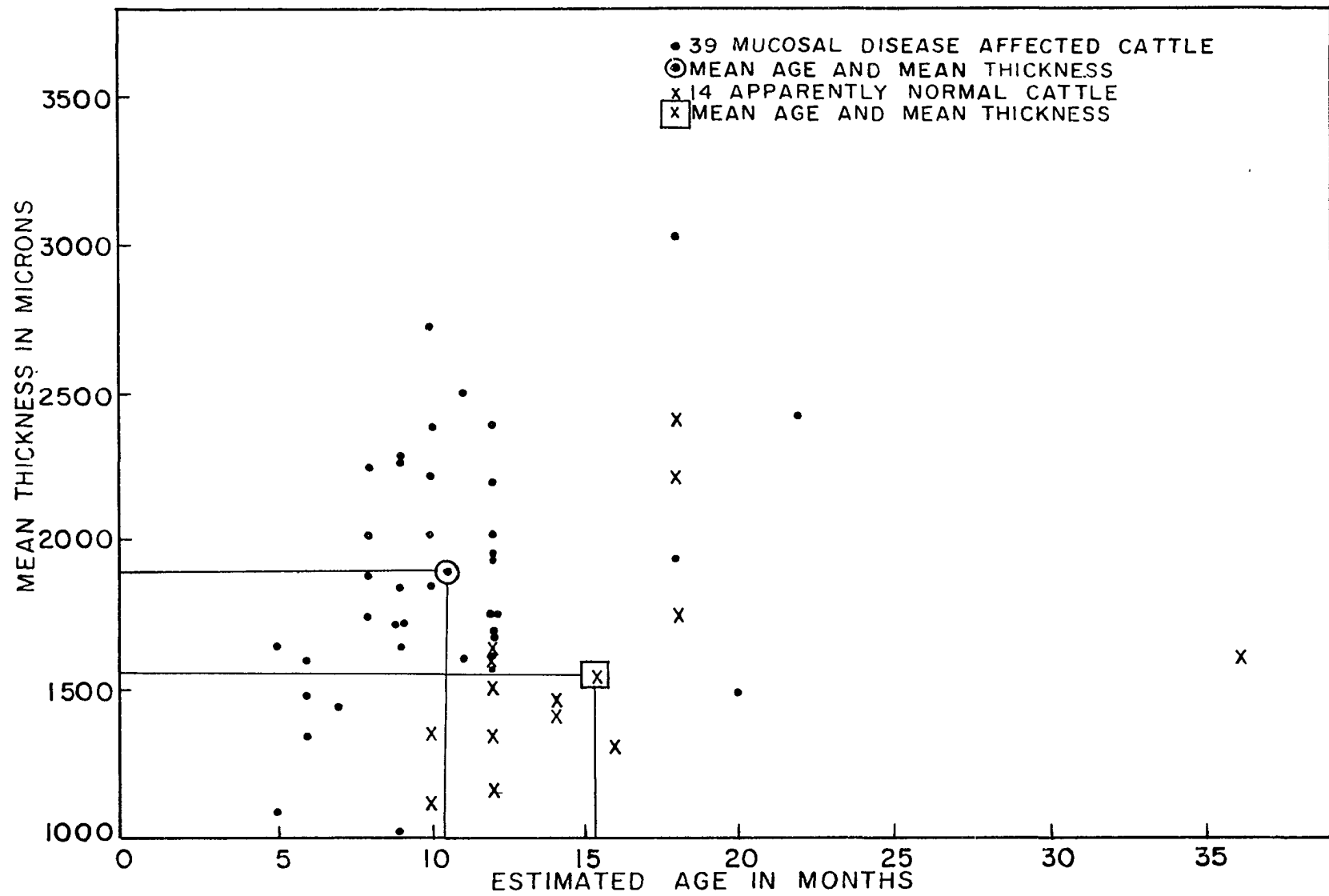
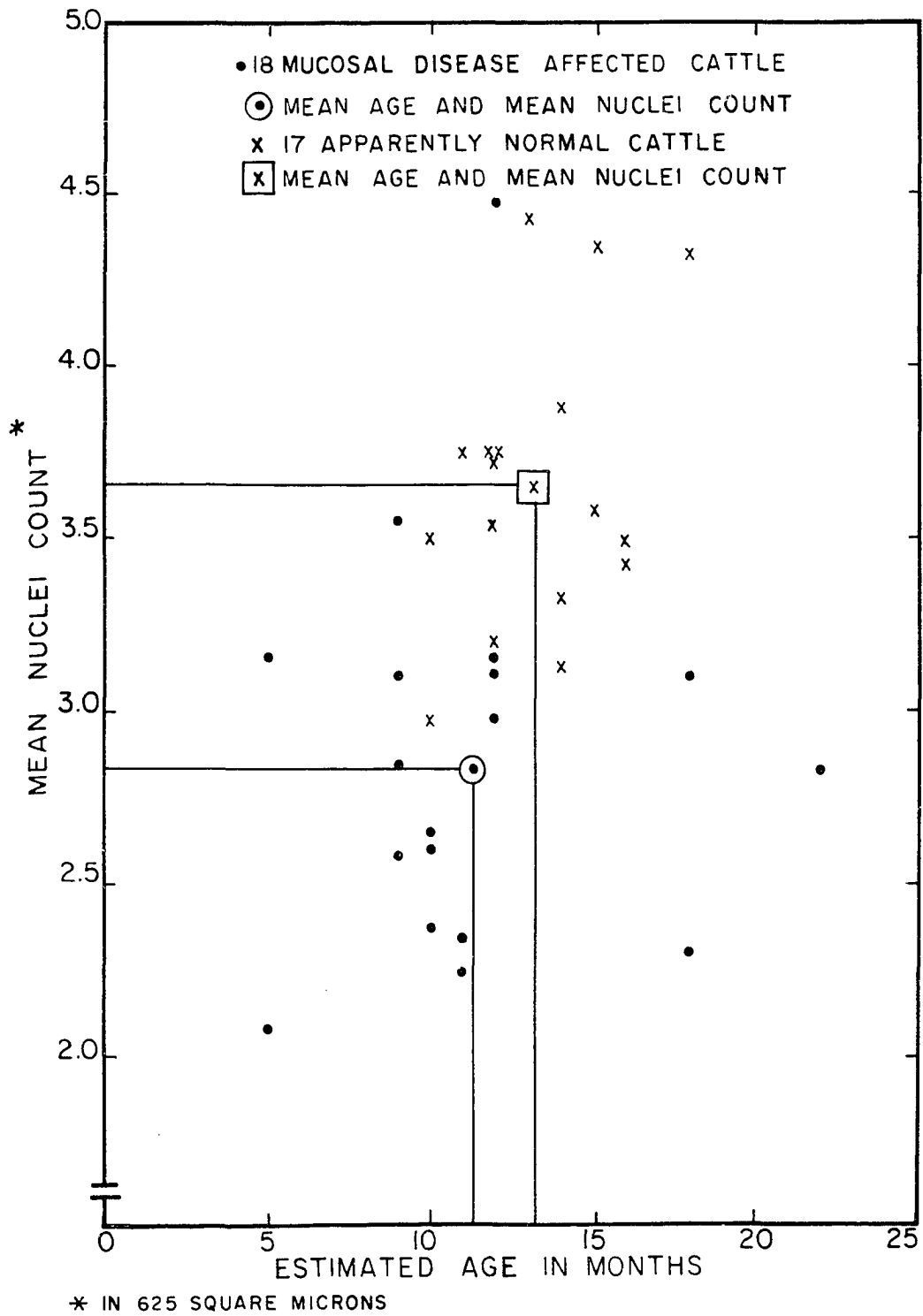


Figure 30. Zona fasciculata-zona reticularis midzonal nuclei counts plotted against the estimated ages of the cattle. A low mean nuclei count indicates that large cells were present



was 15.28 months.

Figure 30 shows data on the mean nuclei counts made in the midzone of the zona fasciculata-zona reticularis in the adrenals of 18 mucosal disease affected cattle and 17 normal cattle. The nuclei counts were plotted against the estimated ages of the donor cattle in months. The overall mean midzonal nuclei count for the adrenals of the 18 mucosal disease affected cattle was 2.83 nuclei per unit area* and the mean age of the same group of cattle was 11.3 months. The overall mean midzonal nuclei count for the adrenals of the 17 normal cattle was 3.65 nuclei per unit area and the mean age of the same group of cattle was 13.3 months.

Mitotic figures were occasionally observed in adrenal sections from affected cattle, but were seldom observed in adrenal sections from normal cattle. In sections from affected cattle from one to six mitotic figures were routinely observed in the zona fasciculata of a single transverse adrenal section. The mitotic figures were usually observed in the peripheral one third, or one half, of the zona fasciculata. Sections taken from one animal that was destroyed in the early stages of mucosal disease revealed numerous mitotic figures (Figure 31), especially in one quadrant of the adrenal sections. Mitotic figures were infrequent in zones other than the zona fasciculata.

Fatty alterative changes, as seen in the zona fasciculata, extended throughout the adjacent zona reticularis, but tended to be less marked there. The zona glomerulosa showed similar but minimal changes (Figure 32), and in only those adrenals that showed marked fatty alterative

*Unit area was 625 square microns.

changes in the zona fasciculata and zona reticularis. Adrenals that showed little or no fatty change in the zona fasciculata had zona glomerulosa cells with normal appearing cytoplasm.

The width of cortical sinusoids varied in mucosal disease affected adrenals. The width appeared to vary inversely with the degree of hypertrophy of parenchymatous cells that were undergoing fatty alternative changes. Since fatty alternative changes were variable within the same adrenal section, both compression and dilatation of sinusoids were occasionally present in the same section. Widely dilated sinusoids appeared in the zona fasciculata-zona reticularis of approximately one eighth of the affected adrenals examined and gave those sections a pocked appearance (Figures 33 and 34). One to three necrotic parenchymatous cells were observed at the margin of many of the widely dilated sinusoids.

Four of 42 adrenal cortices from affected animals also exhibited rather extensive areas of cell necrosis in a portion of the zona fasciculata-zona reticularis (Figures 35 and 36), but the cell necrosis did not extend into the medulla. In those four adrenals approximately one third of the parenchymatous cells in a single 486X field were necrotic. However, such areas of necrosis involved no more than one tenth of the total cross section of the adrenal.

Necrotic cells of the zona fasciculata and zona reticularis were characterized by cell shrinkage, cytoplasmic eosinophilia and fuchsino-
philia, nuclear pyknosis, and occasional karyorrhexis and karyolysis. In the adjacent zona glomerulosa one to a dozen cells sometimes showed a comparable, or lesser, degree of nuclear pyknosis, but fatty cytoplasmic

Figure 31. Mitotic figures in the zona fasciculata of an adrenal from a mucosal disease affected heifer. X 190. The heifer was sacrificed in an early stage of mucosal disease

Figure 32. Fatty alterative changes in the zona glomerulosa of an adrenal from a mucosal disease affected steer. X 486

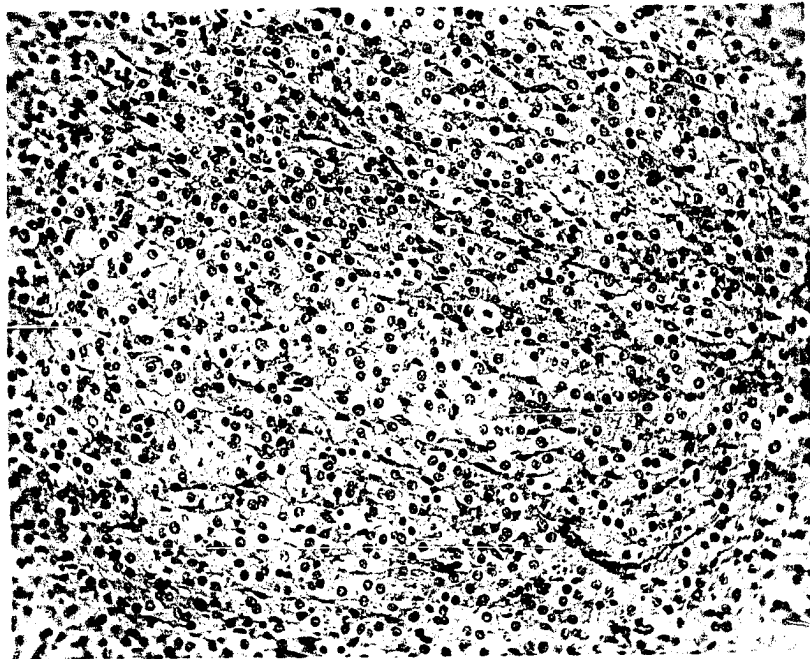
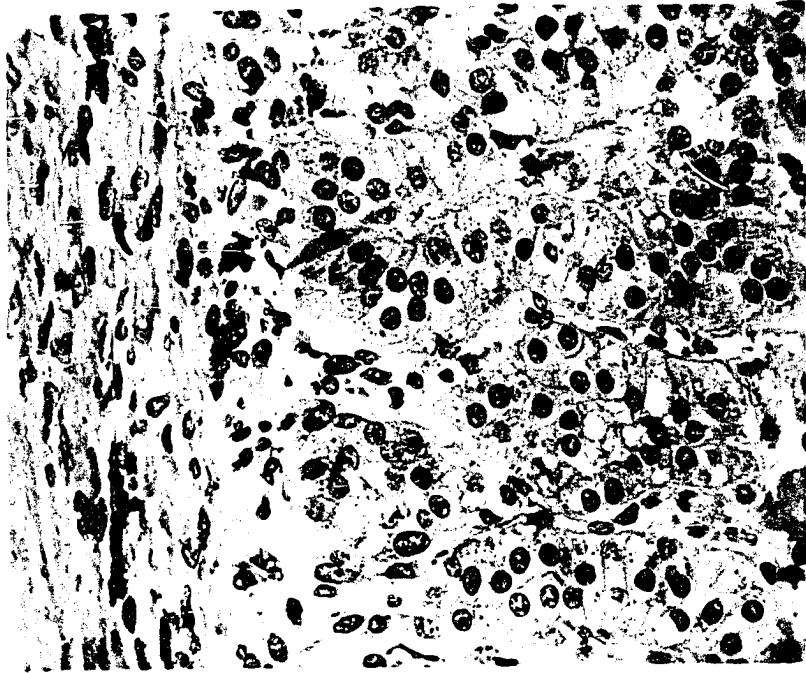


Figure 33. Dilated sinusoids in the zona fasciculata-zona reticularis of an adrenal from a mucosal disease affected steer. X 12

Figure 34. A dilated sinusoid from the adrenal section shown in Figure 33. X 486. Note that fatty alterative changes and nuclear pyknosis are present

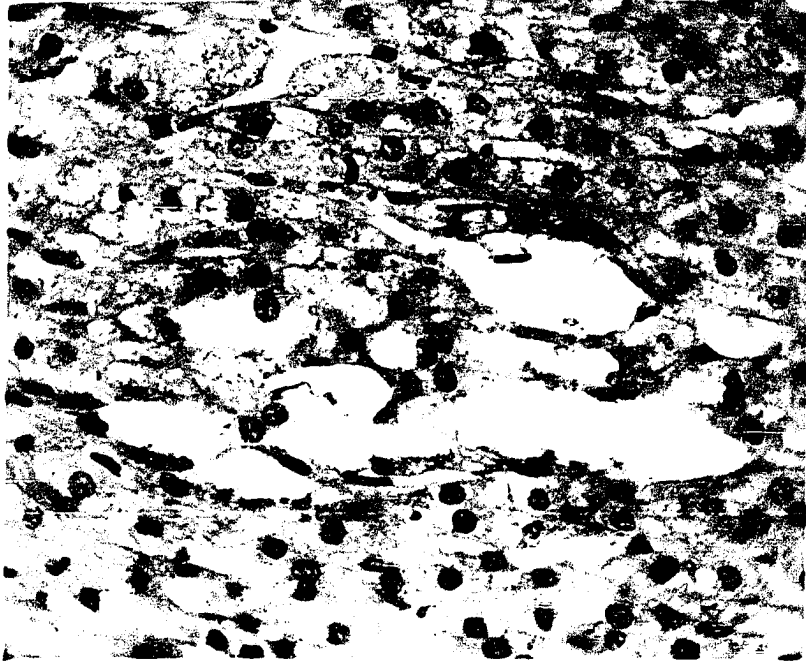
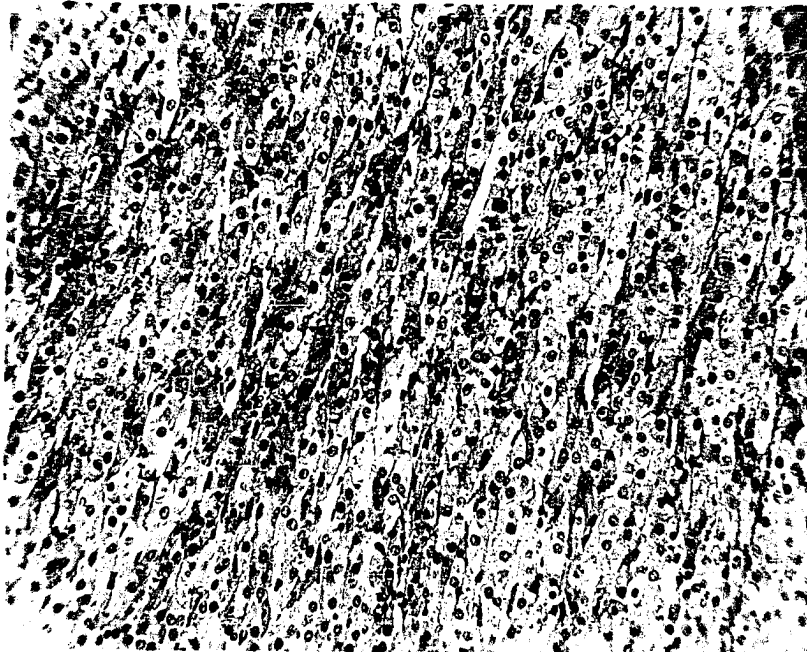
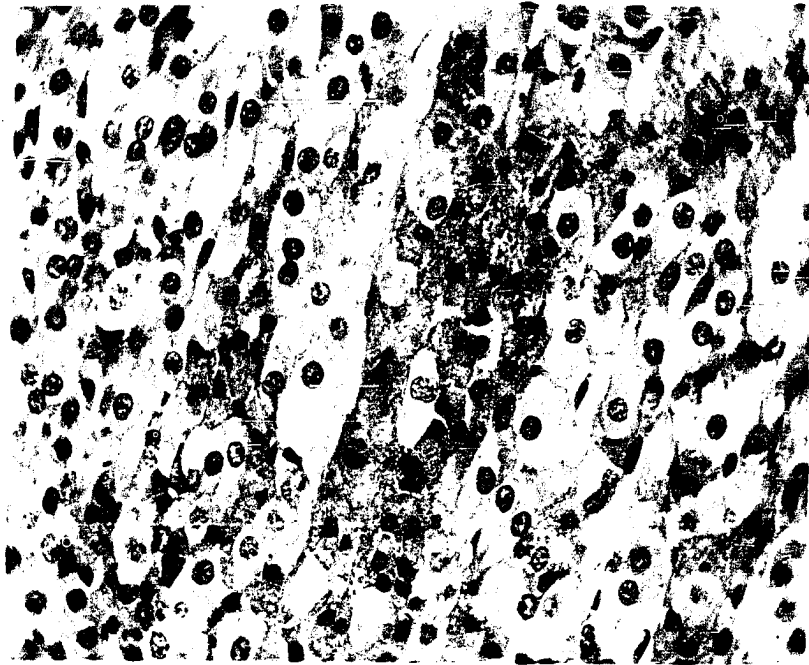


Figure 35. Cell necrosis in the zona fasciculata-zona reticularis of an adrenal from a mucosal disease affected steer. X 190. In the necrotic cells note the increased cytoplasmic staining, cell shrinkage, and nuclear pyknosis. Other cells between the necrotic cells show marked fatty alterative changes

Figure 36. A higher magnification of a portion of the section used for Figure 35. X 486



changes were usually absent or minimal.

Three of the four adrenals that exhibited extensive cell necrosis, as just described, also had extramedullary hematopoiesis present in some part of the section. The latter process occurred among the cords of degenerating or necrotic parenchymatous cells (Figures 37 and 38). The hematopoietic areas were made up predominantly of eosinophils and neutrophils, and immature forms of those granulocytes were numerous. Macrophages were usually present. Large cells having multilobed nuclei, believed to be megakaryocytes, were present in one adrenal (Figure 38). Adrenal sections from three of 20 normal cattle also had extramedullary hematopoiesis. In those adrenals the parenchyma within and adjacent to the hematopoietic area appeared normal.

Histochemical determinations

Frozen sections from adrenals of 10 typical mucosal disease affected cattle were stained with oil red O. Those adrenals all contained large oil red O-stainable lipid globules in their cortices (Figure 39). Adrenals from 15 normal cattle, similarly and simultaneously processed, did not exhibit such globules although the zona fasciculata and zona reticularis did stain a very weak diffuse brownish-red (Figure 40).

The lipid globules in the adrenals of mucosal disease affected cattle were present in all the zones of the adrenal cortex. Quantitatively, the zona fasciculata cells took up more oil red O. The zona reticularis, zona glomerulosa, and zona intermedia followed in order. The lipid

Figure 37. Extramedullary hematopoiesis in the adrenal cortex. From a mucosal disease affected steer. X 96. A, capsule; B, zona reticularis

Figure 38. A higher magnification of a portion of the section shown in Figure 37. X 486. Note the multilobed nucleus present in one large cell. Hematopoiesis is present between the cords of parenchymatous cells

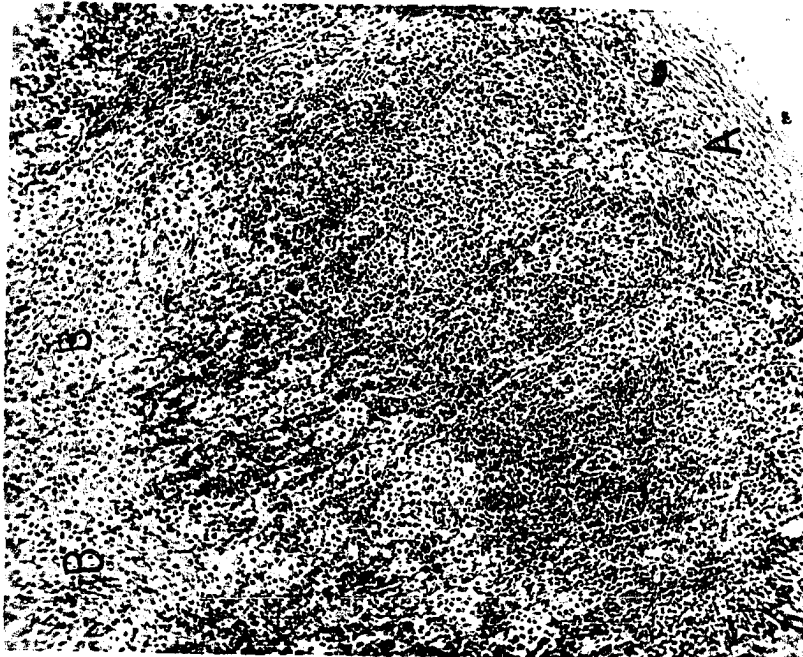
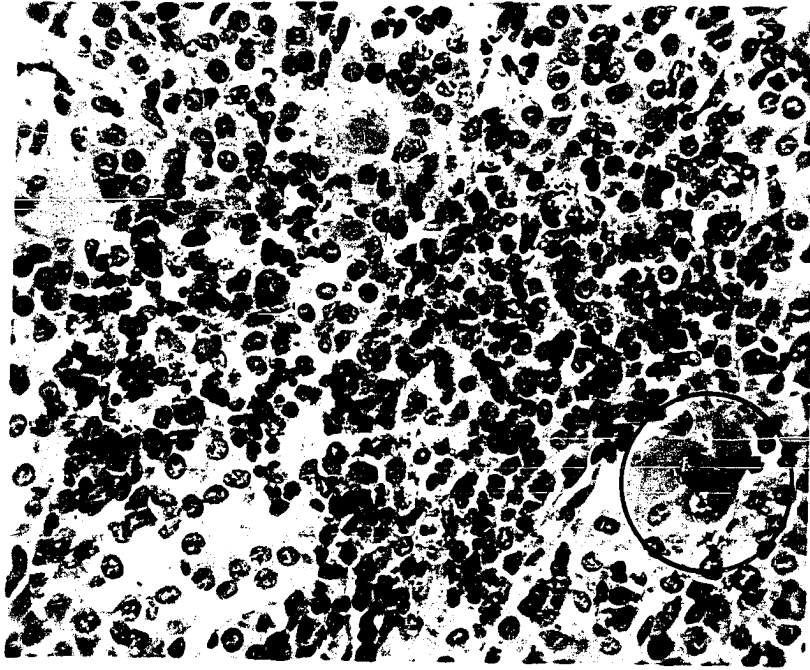
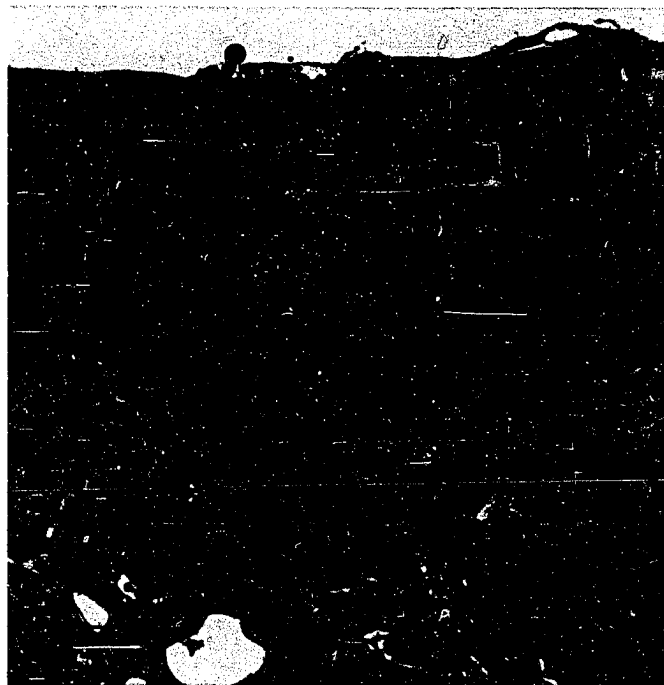
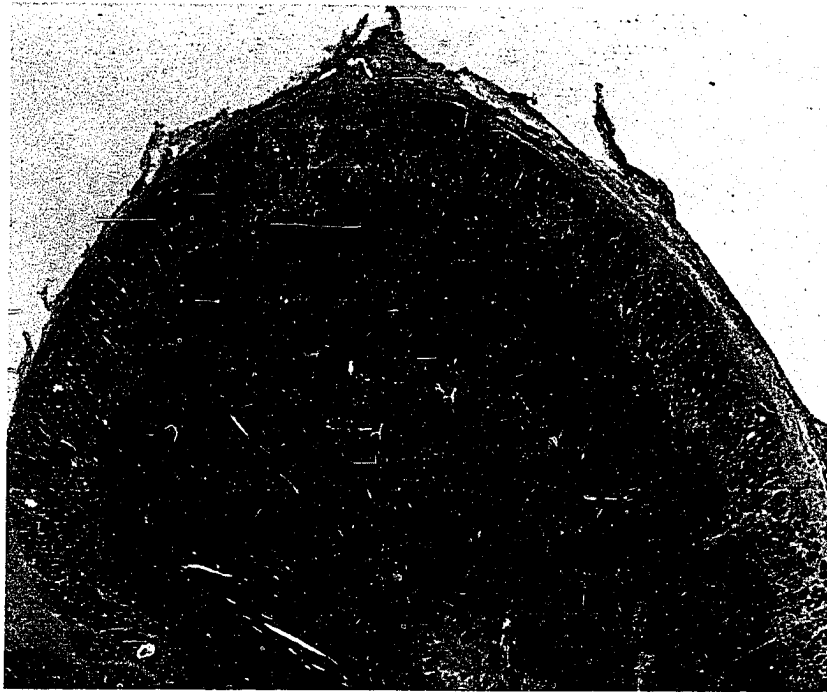


Figure 39. An oil red O stain of an adrenal frozen section. From a mucosal disease affected bull. X 19. Note that oil red O-stainable materials are present in all cortical zones, but are especially prominent in the zona fasciculata and zona reticularis

Figure 40. Oil red O stain of an adrenal section from a normal steer. X 19. Note the faint diffuse brownish red in the zona fasciculata-zona reticularis. Untrimmed fat on the external surface of the adrenal capsule stains a brilliant red. A, zona fasciculata-zona reticularis; B, medulla



globules varied in size from those barely visible under 970X magnification to those that appeared to fill the entire cytoplasmic part of the parenchymatous cells, thus obscuring the nuclei. There was a tendency for the globules in the zona reticularis to be slightly smaller than those seen in the zona fasciculata and zona glomerulosa. The globules in the zona intermedia, or the zone where the zona intermedia would normally be located, were small and not very numerous. The location of oil red O-stainable lipids corresponded to the location of the vacuolation observed in paraffin prepared sections.

All adrenals stained with oil red O were tested also for the presence of cholesterol. Of the 10 mucosal disease affected adrenals tested, five were negative, two showed slight but visible traces of cholesterol, and three gave definitely positive tests. Adrenals that were positive to the cholesterol test exhibited an intracytoplasmic greenish fluorescence in scattered cells of only the zona glomerulosa. Adrenals from 15 normal cattle were negative to the cholesterol test in all cortical zones. Medullated nerves in the medullae of the adrenals of both affected and normal cattle gave positive cholesterol readings and thereby served as a check on the workability of the cholesterol test.

DISCUSSION

The Pituitaries and Adenohypophyses

The mean weight of the intact pituitaries from mucosal disease affected cattle was 0.48 gram less than the mean pituitary weight of the normal cattle. Since the latter cattle were older and heavier, they were expected to have heavier pituitaries (Gilmore, Peterson, and Rasmussen, 1941, Brody and Kibler, 1941).

Casual study of the pituitary weights of the normal cattle and of the affected cattle (Figure 2) showed that the pituitary weights of the normal cattle would fit and predominate the upper end of a single regression line plotted for all pituitary weights. Careful study also revealed the possibility that affected cattle had reduced pituitary weights. That possibility was made more apparent by plotting separate regression lines for the two groups of pituitary weights. Separate regression lines were not plotted in Figure 2 because of dissimilarities of age and weight that existed between the two groups of donor cattle. Hence, it was not definitely determined that pituitaries from affected cattle were reduced in weight, but the possibility was apparent.

A reduction of pituitary weight in mucosal disease affected cattle, if it actually occurs, may be explained in a number of ways. A compensatory atrophy of the pituitary (including the adenohypophysis) may accompany adrenal cortical hypertrophy. It is known that the pituitary and adrenal cortex are physiologically interrelated, and weight changes of the two structures may occur as a physical manifestation of that relation-

ship. Terminal inanition, present in mucosal disease affected cattle, may result in a pituitary weight reduction as was reported by D'Angelo, Gordon, and Charipper (1948) in guinea pigs undergoing starvation.

The fact that the various components of the pituitary were not weighed separately in this study provides an unknown factor in interpreting a possible loss of total pituitary weight. Alterations in the neurohypophysis may influence pituitary weights.

Parenchymatous cell counts of the adenohypophyses of affected cattle indicated that the adenohypophyses of affected cattle had less acidophils and less basophils than did the adenohypophyses of normal cattle (Tables 3 and 4). Assuming that adrenocorticotrophic hormone (ACTH) production is increased to produce the adrenal cortical hypertrophy found in mucosal disease, it may be reasoned that a decrease in the number of acidophils or basophils is related to increased ACTH production.

There is no agreement as to the source of ACTH in the bovine. In species other than cattle certain work (D'Angelo, Gordon, and Charipper, 1948, Marshall, 1951) indicated that ACTH is produced by basophils. Other work (Herlant, 1952, 1953a, 1953b, Barrnett, Siperstein and Josimovich, 1956, Ladman and Barrnett, 1956) indicated that ACTH is produced by acidophils.

Jubb and McEntee (1955b) did not work on the source of ACTH in the bovine, but they reached certain conclusions regarding basophil and acidophil degranulations that are of interest. They were able to relate the two predominant basophilic cells of the bovine adenohypophysis, beta

cells and delta cells, to certain functions. The beta cells were related to thyrotrophic hormone production. The basophilic granules of the delta cells were related to sex and post-castration time. The possibility of other beta and delta cell functions was not excluded.

There were differences in sex and post-castration time between the two groups of cattle involved in this research problem. Those differences were expected to affect the basophil count. In view of this no attempt was made to explain the differences in the number of basophils present in the adenohypophyses of the two groups of cattle.

Jubb and McEntee (1955b) also observed a degranulation of bovine acidophils as a consequence of the stress of a number of unrelated disease conditions. Those authors commented on the nonspecific nature of that degranulation. Apparently mucosal disease provides a stress that produces a similar degranulation of acidophils. It is considered possible that degranulation of acidophils in mucosal disease is related to the increased production of ACTH. There is also a possibility that acidophil granules are not replaced in the terminal stages of mucosal disease. The scarcity of acidophilic granules may be related to poor nutrition that accompanies terminal inanition (Ershoff, 1952).

The Adrenals

The adrenal capsule

Upon microscopic examination a decrease in the thickness of the adrenal capsule was observed in the adrenals of many mucosal disease affected cattle. However, exceptions were noted. The inner cellular part

of the affected capsules showed the greatest decrease in thickness. It was first reasoned that the capsule thinning was the result of differentiation of inner capsule cells into adrenal parenchymatous cells, a normal process previously reported by Elias (1945). Study of the thinned adrenal capsules found in mucosal disease did not reveal an increased differentiation of inner capsule cells into adrenal parenchymatous cells.

Capsule thinning may have resulted from stretching of the adrenal capsule as the result of adrenal cortical hypertrophy and, to a lesser extent, as the result of cloudy swelling. The loose inner capsule may be more compressible. The observation of capsule thinning was in contrast to the observation of Garm (1949) in his work with nymphomania. In that disease the adrenal capsule was observed to be thickened.

Lymphocyte and eosinophil infiltration

Lymphocytic infiltrations, with a few eosinophils, were found in many trabecula-capsule junctions in the adrenals of 18 of 23 affected cattle destroyed with electricity. A similar cellular infiltration was found in all adrenals from 18 cattle that died of mucosal disease. These observations are interpreted to mean that lymphocytic infiltrations of the adrenals occur in the advanced stages of mucosal disease, but are more pronounced in the terminal phases.

Three possibilities were considered in an effort to explain the presence of lymphocytes in the adrenals:

- (1) Rather extensive areas of necrosis were observed in four of 42 pairs of adrenals. Similar areas could have been present in other adre-

nals since serial sections were not made. It is known that lymphocytes are commonly found in areas of inflammation. Possibly the lymphocytes were in the adrenal as a response to inflammation incited by necrotic parenchymatous cells. Nevertheless, inflammation of that nature probably would have attracted more neutrophils than were observed.

- (2) Perivascular cuffing of lymphocytes is the typical and sometimes the only visible expression of certain virus diseases affecting the central nervous system. The perivascular infiltration seen in the adrenals of mucosal disease affected cattle may be the result of a virus infection that produces a similar perivascular cuffing. The etiology of mucosal disease is presently unknown. It may be caused by a virus.
- (3) It is of interest that the infiltrating cells observed in the adrenal, namely lymphocytes and eosinophils, are known to be present in the normal bovine blood stream. Furthermore, the blood levels of these cells are known to be influenced or regulated by adrenocortical steroid hormones commonly referred to as glucocorticoids. In mucosal disease a relative lymphopenia and an eosinopenia were observed in the early stages of mucosal disease by Trapp*. No conclusion was reached as to the possible relationship of the early lymphopenia and eosinopenia to the infiltrating lymphocytes and eosinophils found in the adrenal. Nevertheless, it appears that a

*Trapp, Dr. A. L., Iowa State, Ames, Iowa. Data on blood studies in mucosal disease. Private communication, 1958.

relationship may exist.

It should be mentioned here that eosinopenia, lymphopenia, and lymphoid atrophy are all influenced by glucocorticoids. Lymphoid tissue alterations were observed in mucosal disease (Ramsey, 1956), especially in Peyer's patches, and may have been influenced by glucocorticoids.

Bovine malignant head catarrh

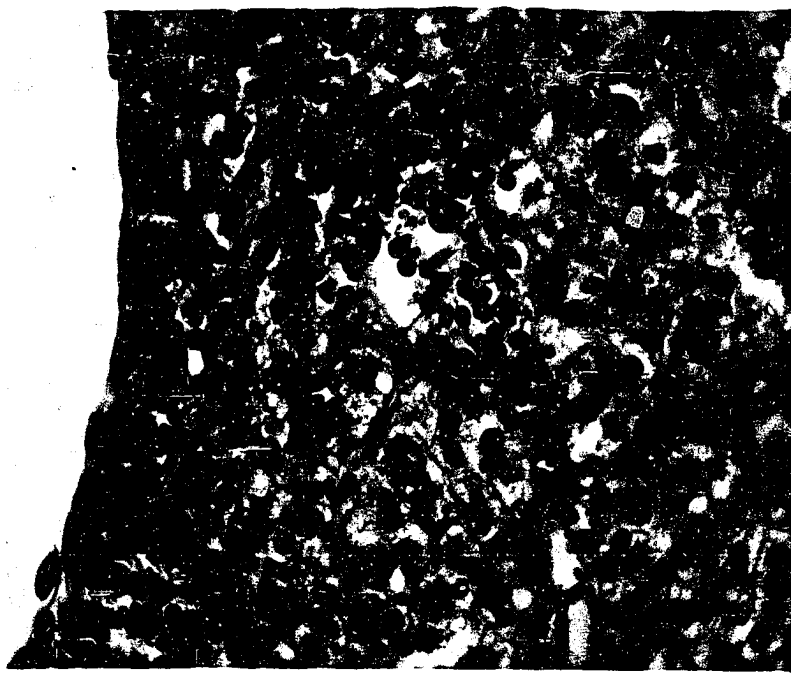
During a consideration of the etiology of mucosal disease adrenal sections were studied from four cattle with malignant head catarrh, a disease thought to be caused by a virus. Adrenal sections from three of the four cattle exhibited a lymphoid-macrophage type of cell infiltration, (Figures 41 and 42) as described by Plowright (1953). The infiltration involved the adrenal capsule, the entire cortex (but especially the zona glomerulosa), and the adrenal medulla. Parenchymatous cell necrosis and macrophages were observed in the zona fasciculata. The infiltrating cells appeared to be intermediate in form between lymphocytes and macrophages. A differentiation of the adrenals from mucosal disease and malignant head catarrh was readily made on the basis of differences in morphology that existed between infiltrating cells, and on the basis of the distribution of infiltrating cells.

Adrenal hemorrhage

Variable numbers of petechial hemorrhages were present in the adrenals of cattle that died of mucosal disease. Similar hemorrhages, although less numerous, occurred in the adrenals of cattle that were moribund when destroyed. Subepicardial and subendocardial hemorrhages,

Figure 41. Lymphoid-macrophages present in the capsule of an adrenal from a heifer with malignant head catarrh. X 486. Note that many of the cells are intermediate in form between lymphocytes and macrophages. A small artery, sagittally cut, is present in the capsule

Figure 42. A view from the medulla of the same adrenal section shown in Figure 41. X 486. Note again the presence of lymphoid-macrophages



often associated with terminal anoxia in cattle, were observed concurrently with most adrenal hemorrhages. Adrenal and heart hemorrhages occurring with mucosal disease probably are the consequence of terminal anoxia.

Hyaline-like droplets in sinusoids of adrenals from mucosal disease affected cattle are believed to be a blood serum component since they are present within blood vessels. The increased number of hyaline-like droplets found in cattle that died of mucosal disease may be related to the marked dehydration and hemoconcentration noted by Trapp during the later stages of mucosal disease.

Fatty alterative changes

In most paraffin prepared sections from mucosal disease affected adrenals, fatty alterative changes, manifested as a cytoplasmic vacuolation, were observed in all zones of the adrenal cortex. Cytoplasmic vacuolation was especially marked in the zona fasciculata and zona reticularis. Oil red O-stained frozen sections were made from 10 mucosal disease affected adrenals. They demonstrated that proportionate quantities of oil red O-stainable materials, presumably fats or lipids, were present in the same cortical zones exhibiting cytoplasmic vacuolation. It is believed that the vacuolation observed in paraffin prepared sections was related to the presence of lipids within parenchymatous cells.

Not only were oil red O stains made on frozen sections from 10 mucosal disease affected adrenals, but cholesterol determinations were made on companion sections cut at the same time from the same tissues. Five

of the same 10 adrenals gave positive cholesterol readings in the zona glomerulosa, although the strength of the reaction varied. It is believed that functional specialization of the adrenal zona glomerulosa may be responsible for positive cholesterol determinations occurring only within the zona glomerulosa.

The accumulation of fats or lipids within the bovine adrenal, with or without cholesterol accumulations, has been reported in many other abnormal conditions in cattle. Fats or lipids accumulate in the adrenal in bovine ketosis (Shaw, 1948, 1949) and in parturient paresis (Garm, 1950). Likewise, lipids and cholesterol accumulate in the adrenals of calves as a result of experimental procedures that could be expected to upset electrolyte metabolism (Weber, Pritchard, and Sellers, 1956). Weber, Bell and Sellers (1958) produced intracellular coarse lipid droplets and cholesterol accumulations in the adrenals of calves by the administration of a chlorinated hydrocarbon. Bell and Weber (1959) observed that lipids accumulated in the adrenals of cows undergoing the stress of lactation and pregnancy.

It appears from study of the references just quoted in the preceding paragraph that accumulation of lipids and cholesterol in the bovine adrenal are common responses to various forms of stress. Of the two responses the accumulation of lipids was more consistently reported. In some cases cholesterol determinations were not made. It should be noted at this point that the Schultz cholesterol test was shown to be relatively insensitive to the presence of appreciable amounts of cholesterol (Reiner, 1953).

The interpretation of the significance of oil red O-stainable lipids and positive cholesterol determinations in adrenal sections depends on the species of animal under consideration. The bovine adrenal is considered to be low in cholesterol (Glick and Ochs, 1955) and lipid-poor (Verne and Hebert, 1952). The latter authors theorized that adrenal hormones are not stored in large quantity in lipid poor adrenals as precursors (meaning in the form of lipids or cholesterol), but are secreted immediately upon production.

Weber, Pritchard, and Sellers (1956) interpreted lipid accumulation and positive cholesterol readings, produced experimentally in the zona glomerulosa of calves, to indicate the inactivation of involved cells. Bell (1956) also theorized that inactivation of cortical cells might result in a piling up of cholesterol in the bovine adrenal.

Lipid accumulation, and to a limited extent cholesterol accumulation, appears to be associated with fatty alterative changes observed in the adrenals of cattle with mucosal disease. Variations in cholesterol readings are believed to be related to the various stages of mucosal disease under investigation and to the limited sensitivity of the modified Schultz cholesterol test.

Marked parenchymatous cell necrosis was observed in the adrenals of four cattle with mucosal disease. All of those adrenals showed marked fatty alterative changes. It is believed that adrenal parenchymatous cell necrosis developed as a termination of fatty alterative changes in those four adrenals.

Extramedullary hematopoiesis is not considered a significant finding

since it appeared in the normal adrenals as well as in mucosal disease affected adrenals.

Adrenal cortical hypertrophy

An increase in adrenal weight occurred in the group of mucosal disease affected cattle (Tables 1 and 2). Moreover, in most mucosal disease affected adrenals the measured zones were increased in thickness and the parenchymatous cells of the two zones of the adrenal cortex were hypertrophied. Increased adrenal weight and increased zone thicknesses are logical consequences of cellular hypertrophy and limited cloudy swelling. The presence of numerous mitotic figures in the adrenal of one animal killed in the early stages of mucosal disease points out the possibility that an early hyperplasia may precede cellular hypertrophy.

Tepperman, Engel, and Long (1943a) pointed out that increased adrenal weight did not necessarily indicate increased adrenal cortical activation. However, Deane, Shaw, and Greep (1948) were able to relate adrenal cellular hypertrophy in the zona glomerulosa of the rat to increased functional activity. Bell (1956) suggested too that the size and weight of the adrenal are important considerations as measures of adrenal activity. Since adrenal cortical hypertrophy appears to be an almost universal response in many species of animals following stress, it is considered that increased adrenal weight, increased zone thickness, and cellular hypertrophy indicate an increased adrenal activity in mucosal disease.

Various factors or agents causing adrenal cortical hypertrophy in

laboratory animals were considered in an effort to explain the similar observation in mucosal disease. Those factors and agents included bacterial infections (Deansley, 1931), inanition (Selye, 1936), anoxia (Langley and Clark, 1942), estrogens (Bourne and Zuckerman, 1940), toxic substances (Tepperman, Engel, and Long, 1943a), and high protein diets (Tepperman, Engel, and Long, 1943b).

Although one or more of the factors or agents just listed may have influenced adrenal alterations in mucosal disease, none was specifically incriminated. Etiologically important bacteria were not isolated. Inanition was considered to be only a short-term terminal factor in adrenal alterations. Conclusions were not reached regarding the influence of estrogens, toxic substances of unknown origin, or high protein diets. The latter three factors or agents are considered as possibly important factors, but could not be evaluated in relation to this study.

A precise interpretation of the physiological or pathological significance of adrenal cortical alterations could not be made since it is not known whether varied functions of the adrenal cortex are mediated equally by all parts of the cortex (Maximow and Bloom, 1947). The preponderance of evidence seems to indicate that the zona glomerulosa hormones are associated largely with electrolyte balance whereas the zona fasciculata hormones are concerned largely with glucose metabolism.

If one accepts that the zona glomerulosa hormones are largely associated with electrolyte balance within the body, then the zona glomerulosa alterations observed may be related to electrolyte imbalance in mucosal disease. Mucosal disease affected calves are dehydrated to

an extreme degree. Electrolyte imbalance may accompany the dehydration.

If one accepts that the zona fasciculata-zona reticularis is concerned with glucose metabolism, then the alterations observed there may be related to the marked increase of blood glucose levels noted by Trapp in many advanced cases of mucosal disease. Assuming that alterations observed in the zona glomerulosa and the zona fasciculata-zona reticularis are related respectively to electrolyte metabolism and glucose metabolism, it is impossible to say whether the alterations are the cause or the effect. It is interesting to note that adrenal enlargement is present in Domsiekte and bovine ketosis, two other ruminant diseases in which altered glucose metabolism occurs.

The Etiology of Mucosal Disease

As previously indicated, the etiology of mucosal disease is not understood. Nor did study of the histopathology of the adrenal cortex and adenohypophysis elucidate its etiology. The alterations observed may be those of a nonspecific stress response, a response bearing resemblance to responses observed in bovine ketosis, milk fever, nymphomania, and other experimentally produced cattle diseases. Conversely, the same alterations may be related to the stress that occurs in the course of a disease produced by a specific etiological agent. In the light of present knowledge regarding Iowa mucosal disease both possibilities are believed to be worthy of consideration.

SUMMARY

1. The adrenals from 42 typical cases of bovine Iowa mucosal disease were obtained. In addition the pituitaries of 25 of the same animals were collected. The study was primarily confined to histopathological study of the adrenal cortices and adenohypophyses. Total adrenal weights and pituitary weights were determined on some animals.
2. Adrenals and pituitaries of 18 apparently normal cattle were studied and compared with similar tissues from mucosal disease affected cattle.
3. It was believed that the weights of pituitaries from mucosal disease affected cattle were reduced. This was not proved. Differences in ages, weights, sex, and breed prevented fair comparison of the tissues of affected cattle and normal cattle, a difficulty that persisted throughout the study.
4. Microscopic examination of adenohypophyses from mucosal disease affected cattle showed that the adenohypophyses were congested. In addition, differential parenchymatous cell counts of 20 of the adenohypophyses showed that they contained less basophils, less acidophils, and more chromophobes than did the normal adenohypophyses.
5. Adrenal parenchymatous cells, found in the zona glomerulosa and in the midzone of the zona fasciculata-zona reticularis of affected cattle, were larger than in normal cattle. The two cortical zones that contained those cells were increased in thickness. The adre-

- nals from mucosal disease affected cattle were increased in weight.
6. The physical alterations of the adrenals were interpreted to indicate that cellular hypertrophy occurred in the adrenals in mucosal disease. Early parenchymatous cellular hyperplasia was considered a possible early adrenal alteration.
 7. Microscopic observation indicated that the adrenal capsules were reduced in thickness in most mucosal disease affected adrenals. Exceptions were observed. Measurements were not made of adrenal capsule thickness.
 8. All adrenals from 19 cattle that died of mucosal disease contained a lymphocytic infiltration in many trabecula-capsule junctions. A similar infiltration was present in the adrenals of 18 of 23 affected cattle that were killed with electricity. In all adrenals containing lymphocytic infiltrations, occasional eosinophils were present among the lymphocytes.
 9. In most adrenals from mucosal disease affected cattle, fatty alternative changes were present in the zona fasciculata and the zona reticularis. When fatty alternative changes were marked in those two zones, those changes were observed also in the zona glomerulosa and in the zona intermedia, but were less marked there.
 10. Marked but localized parenchymatous cell necrosis was present in adrenals from four cattle with mucosal disease. Mild scattered cell necrosis was observed in many other adrenals from affected cattle.
 11. Adrenals from cattle that were destroyed in the moribund stage of

mucosal disease or that died of mucosal disease contained variable numbers of petechial and ecchymotic hemorrhages that were located predominantly in the peripheral zona fasciculata.

12. Hyaline-like droplets were present in the sinusoids and blood vessels of the adrenals of many cattle with mucosal disease.
13. Oil red O stains made on frozen sections showed that adrenals from 10 mucosal disease affected cattle contained oil red O-stainable lipids in all cortical zones. Those lipids corresponded in location and amount with fatty alterative changes observed in paraffin prepared sections.
14. Frozen sections from the same ten adrenals stained with oil red O were tested for the presence of cholesterol. Five of the ten adrenals gave positive readings.

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